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Institute of Physiology, University of Lund.

The Electrophysiology of the Submaxillary Gland of the Cat.

By

ANDERS LUNDBERG.

Received 9 April 1955.

BAYLISS and BRADFORD (1886) discovered that, during secretion in a salivary gland, a potential could be led off between one electrode at the hilus and another on the surface of the gland. In the submaxillary gland they found that stimulation of the chorda gave an initial hilus positive potential which, however, decreased markedly with continued stimulation even to such an extent that the sign of the potential was reversed. The possibility of the potentials being due to vasomotor changes in the gland was ruled out for the reason that the vasodilatation on chorda stimulation was not much affected by atropine enough to abolish the secretory potentials (BAYLISS and BRADFORD 1886). BRADFORD (1887) also rejected the possibility that the flow of saliva could be the cause of the electrogram, because he found that the potential changes were not affected after the compression of Wharton's duct. Moreover, the outflow of saliva after removal of the obstruction did not produce an electrogram. BRADFORD therefore concluded that the electrogram originated from the glandular cells.

BAYLISS and BRADFORD (1886) came to the conclusion that the chorda electrogram of the submaxillary gland had two components, one hilus positive, and one hilus negative. They associated secretion of salt and water with hilus positivity and secretion of a viscid saliva with hilus negativity. LANGENSKIÖLD (1941) presented further evidence pointing in the same direction, whereas v. HARREVELD (1930 c) rejected this idea. Detailed references to

earlier electrophysiological experiments on salivary glands are found in the papers by v. HARREVELD (1930 a, b, c) and in the monograph by LANGENSKIÖLD (1941).

With the advance of electrophysiological technique it would appear justified to attempt further experiments to determine the origin of the electrogram of the salivary glands. The first part of this paper concerns the external electrogram of the submaxillary gland with special reference to contributions from the ducts and in the second part potentials will be described recorded with glass capillary microelectrodes inserted into the gland. Some of the findings have been reported earlier (LUNDBERG, 1954 a, b).

Methods.

The cats were anesthetized with chloralose (70 mg/kg intravenously). The submaxillary gland was freed from the surrounding tissue with the exception of the nerves, duct, artery and vein. The sublingual gland along the duct of the submaxillary gland was removed. A cannula was inserted into the duct of Wharton, permitting observation of the flow of saliva.

For recording of the external electrogram, the gland was placed on a perspex tray which was lifted so that the only connections between the gland and the rest of the animal were the duct, nerves and blood vessels. Ag-AgCl electrodes were used for stimulation and recording. Recording of the electrogram was made with one electrode on the free surface of the gland, and the other either on the duct close to the hilus, or anywhere on the tissue in the region.

For the microelectrode experiments as well the gland was placed on a plastic tray. The connective tissue sheath was carefully removed within a small area. A nylon-net with fine meshes was gently stretched over the gland. The two main obstacles were the connective tissue and the movements of the gland. Obviously the interstitial connective tissue could not be removed, and caused frequent breakage of electrodes. It proved an advantage to use only young cats with a weight of about 2 kg. Pulse-synchronous and respiratory movements in the resting gland sometimes made the experiments impossible but usually the major obstacle were the movements occurring on stimulation of the secretory nerves. They were due in part to changes in blood flow through the gland, but even after clamping the artery of the gland some movements of the gland persisted, most likely due to activation of contractile elements. Before the actual examination with microelectrodes was started, the gland surface was explored to find a region with minimal movements.

Conventional glass capillary microelectrodes as described by LING and GERARD (1949) and NASTUK and HODGKIN (1950) were used. They were filled with 3 M KCl and had resistance from 2 to 20 mΩ. How-

ever, in some control experiments the electrodes were filled with 0.154 M KCl or NaCl. For recording, a dc-amplifier with a cathode follower (RCA 954) as input was used. The grid current was less than $1 \cdot 10^{-11}$ A. The microelectrode was connected to the input stage through an Ag-AgCl electrode, and on the surface of the gland a large Ag-AgCl electrode was connected to earth via small resistances, through which a calibration voltage could be applied. The microelectrode assembly was carried on a Zeiss slide micromanipulator. A dissecting microscope with magnification up to 160 times was used for dissection and to guide the insertion of the microelectrode.

In the records reproduced chorda stimulation is marked by a signal in the lower and sympathetic stimulation by a signal in the upper edge of the film.

Results.

1. External electrogram of the submaxillary gland.

Fig. 1 shows the potential recorded with one electrode on the hilus and another electrode on the free surface of the gland. These records are similar to those published by LANGENSKIÖLD. With stimulation of the chorda at 25/sec. (upper record), hilus positivity appears after a latency of about 0.3 sec. With continu-

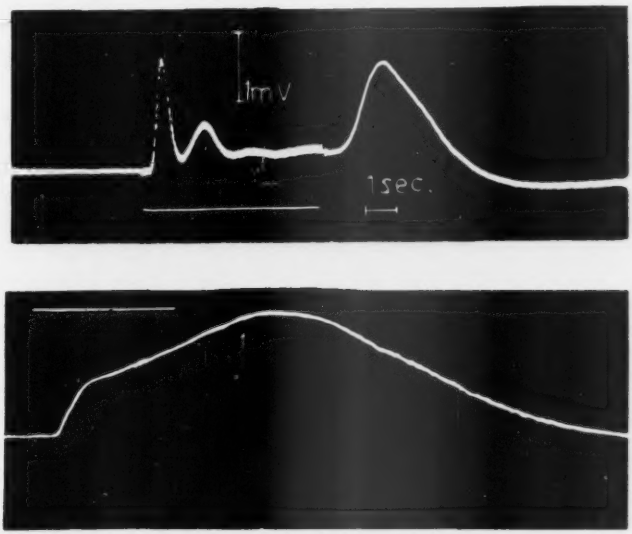

Fig. 1. Upper record: chorda electrogram. Lower record: sympathetic electrogram. Duration of stimulation at 25/sec. shown by horizontal white line.

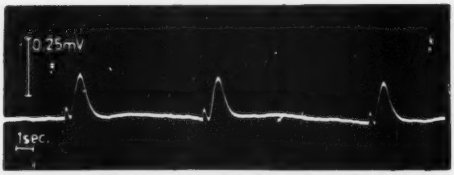

Fig. 2. Electrogram resulting from single shock stimulation of the chorda.

ous stimulation, there is a rapid decrease of potential, followed by a smaller hilus positive potential. Thereafter there is hardly any change of the potential until after the interruption of stimulation, when a large hilus positive potential is recorded. The initial hilus positive potential has been found in all experiments; it was sometimes small but it was never absent. In the great majority of the experiments the latency was 0.2—0.3 sec., but could be as long as 0.6 sec. (Fig. 5). Fig. 2 shows that single shock stimulation of the chorda causes a hilus positive potential which lasts about one sec. The lower record in Fig. 1 shows a typical potential appearing on stimulation of the sympathetic nerve. After a latency of about 0.8 sec., a hilus positive potential appears. This potential has a slower rate of rise than when the chorda was stimulated. Later there is again a slower increase of the potential continuing after the interruption of stimulation, thereafter only to return slowly to the baseline. In the sympathetic electrogram as well the initial hilus positive potential is a constant feature found in all experiments. The latency may in exceptional cases be as short as 0.4 sec. and the rate of establishment was occasionally almost as fast as when chorda is stimulated. Usually the latency was between 0.6 and 1 sec. A potential change was never recorded from the gland on single shock stimulation of the sympathetic nerve.

a) *Variations in the chorda electrogram.*

The late phase of the chorda electrogram seen in Fig. 1 was obtained regularly by LANGENSKIÖLD and described as typical for the submaxillary gland of the cat. VAN HARREVELD (1930 a) working on dogs, found a great deal more variability and described several different types of chorda electrogram. In my experience, there is no constant chorda electrogram in the cat either. The records in Fig. 3 show some different types observed. Record A shows one type fairly often found. After the initial

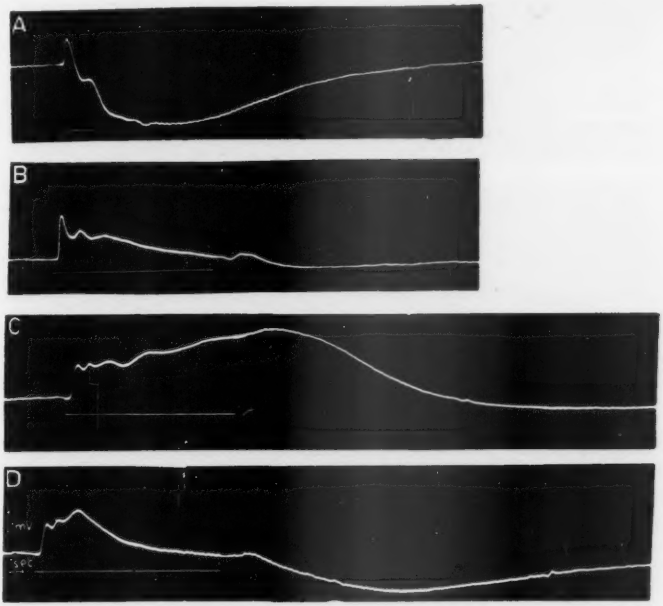

Fig. 3. Variation of chorda electrogram. Records C and D are from the same gland and were obtained at an interval of one minute.

hilus positivity, the potential decreases so that the hilus becomes markedly negative. The second hilus positive potential appears as a notch on this slowly increasing hilus-negativity. After the end of stimulation the potential slowly returns to the baseline, at a rate that is rather similar to that found after stimulation of the sympathetic nerve. Record B in Fig. 3 is similar to the one in Fig. 1 except that the positive overshooting is smaller. This overshooting may also be entirely lacking or have the opposite sign. Record C in Fig. 3 shows another type of potential in which there is hardly any decrease of the initial hilus positive potential, but a slow ascent of the potential continuing even after the end of stimulation. This potential is very similar to the sympathetic electrogram. Such a small degree of decreasing initial positivity was found only exceptionally, but the later slow ascent was not so uncommon. Record D in Fig. 3 was obtained one minute after record C, without any stimulation in the intervening period. The

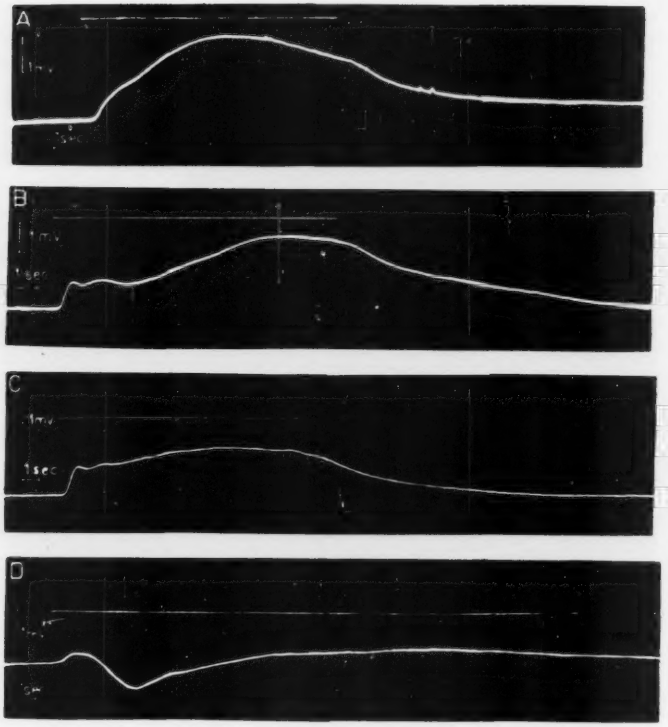

Fig. 4. Variation of sympathetic electrogram.

late slow potential shift now has the opposite sign. Thus, there is a considerable variability in the electrogram of different glands, and appreciable variations are often found in an individual gland. The late course of the chorda electrogram may be either a slow positivity as in the sympathetic electrogram, a slow negativity with about the same time course as this positivity, or absence of any more marked shifts of potential.

b) *Variations in the sympathetic electrogram.*

The sympathetic electrogram from different glands shows less variations as far as the sign of the potential is concerned. In the great majority of experiments there is, following the faster initial hilus-positive potential, only a slow positive potential, but the

time course of this potential can vary considerably. The relation between the magnitude of the two phases may vary considerably; often the sympathetic electrogram is dominated by the second phase, in other cases, only a very small slowly rising potential is superimposed on the initial potential. Fig. 4 demonstrates to some extent the variability of the sympathetic electrogram; it is seen that the second phase appears after a time that is rather variable. In record B, it occurs 4 sec. after the onset of the rapid potential shift, whereas in record A the slow potential begins only 2 sec. after the start of the first. It can be observed in Fig. 4 that the hilus-positive potential resulting from sympathetic stimulation starts to decline before the end of stimulation. This decline of potential is typical for the sympathetic electrogram but the duration of repetitive stimulation necessary varies in different animals. Sometimes the potential starts to decline already after 6—7 sec., sometimes only after 30 sec. On the whole, although the sympathetic electrogram qualitatively is much more constant than the chorda electrogram, great quantitative differences are found. There are not only differences from animal to animal, but even in the individual animal there is seldom a reproducible sympathetic electrogram. In Fig. 4, record C, is also shown an "abnormal" sympathetic electrogram found only on a few occasions.

c) *Influence of previous stimulation on the electrogram.*

Earlier authors (v. HARREVELD, LANGENSKIÖLD) have described that the electrogram changes characteristically after a period of stimulation. They both found that repeated periods of chorda stimulation made the chorda electrogram more positive. I have confirmed this observation, but also observed that after sympathetic stimulation the chorda electrogram is more profoundly changed. One type of change is illustrated in Fig. 5, the chorda electrogram in record A has a very marked hilus-negative slow component. This component did not change sign with repetitive periods of chorda stimulation but 30 sec. after the end of a period of sympathetic stimulation lasting for 10 sec. the chorda electrogram was very much changed as seen in record B. Record C demonstrates the return of the hilus-negative component 12 minutes after record B was taken. Another type of change in the chorda electrogram specifically resulting from a period of sympathetic nerve stimulation is the appearance of a

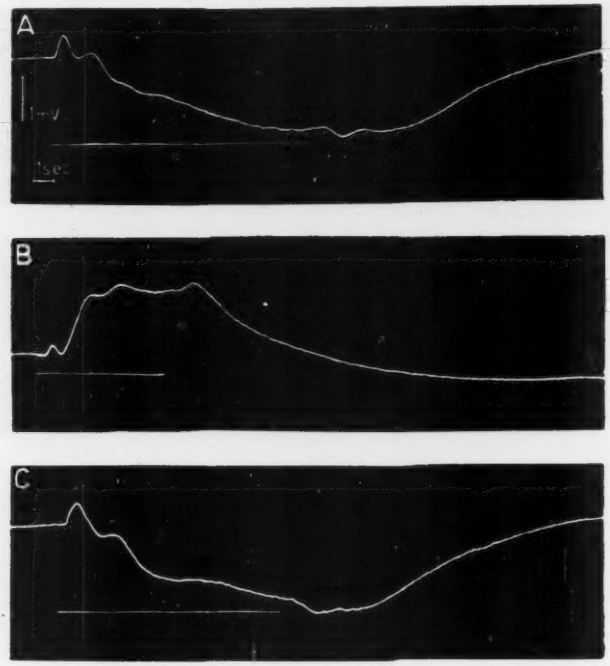

Fig. 5. Variation of chorda electrogram. In the interval between records A and B, the sympathetic nerve was stimulated for 10 secs. Record C obtained 12 minutes later.

large hilus-positive overshooting after the end of chorda stimulation.

LANGENSKIÖLD described that if the chorda was stimulated repetitively during longlasting stimulation of the sympathetic nerve the result was a decrease in the hilus-positive sympathetic component. I have confirmed this observation in some experiments, one of them illustrated in the upper record of Fig. 6. The decrease of hilus-positive potential appears after a latency which is only slightly longer than the latency of the initial hilus-positive component of the chorda electrogram. The lower record in Fig. 6 confirms LANGENSKIÖLD's finding that stimulation of the sympathetic nerve during chorda stimulation does not change the electrogram.

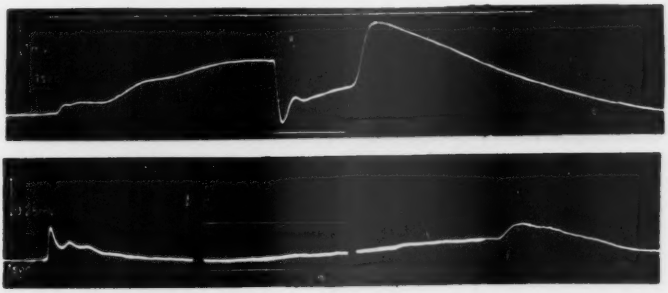

Fig. 6. Upper record shows effect of chorda stimulation during stimulation of the sympathetic nerve. Lower record shows the effect of sympathetic stimulation during stimulation of the chorda.

d) *Records from the duct of the submaxillary gland.*

When recording from a polyethylene catheter or a thin glass capillary tubing, inserted through the main duct, so that the tip is inside the hilus of the gland in one of the larger ducts, stimulation of the secretory nerves resulted in a change of potential. A typical record can be seen in Fig. 7. Downward deflection of the beam denotes that the duct electrode becomes negative relative to the other electrode outside the duct close to the hilus of the gland. In the upper record can be seen how, after a latency of 0.6 sec., the electrode in the duct becomes negative. After about 2 sec. this negativity reaches a plateau. Thereafter it increases only slowly, but this slight increase continues also after the end of stimulation. After having reached the maximum, there is only a slow decline of potential to the baseline. With stimulation of the sympathetic nerve, the potential appears after a longer latency and has a slower rate of rise. It reaches a maximum during sympathetic stimulation and declines slowly irrespective of whether or not the sympathetic nerve is stimulated. After those records had been obtained the catheter was withdrawn from the gland, so that the tip was 6 mm oral to the hilus. Now there was no change of potential during stimulation of either the chorda or the sympathetic nerve. The shape of this duct potential may vary considerably in the same experiment. Characteristic differences between chorda and sympathetic potential are that sympathetic potentials appear after a longer latency and ultimately decline, even if stimulation is continued (as the slow phase of the sympathetic electrogram). This internal duct negativity is sometimes preceded by slight positivity, and in those cases it has also

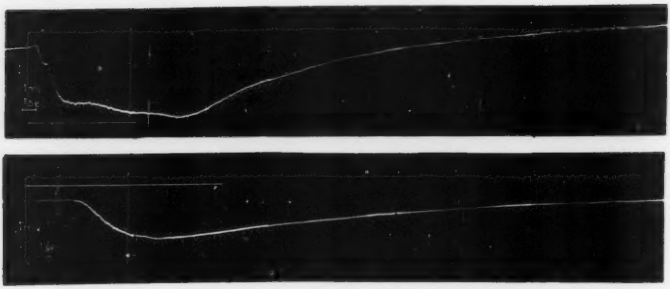

Fig. 7. Potential recorded between the tip of a catheter inserted through the external duct to a position 6 mm inside the hilus and another electrode outside the duct at the hilus of the gland. Upper record chorda stimulation. Lower record sympathetic stimulation. Downward deflection denotes negativity of duct lumen.

been found that when the electrode is withdrawn from the hilus, so that the tip is in the duct well outside the gland, this positivity may persist.

The finding that the negativity of the interior of the duct could not be recorded when the tip of the catheter was at some distance from the gland may indicate that the potential is not caused by the flow alone. To elucidate this question the following experiment was made. All the branches of the common excretory duct were ligated and in one of them a fine polyethylene catheter was inserted permitting injections oralwards into the duct. A second catheter was inserted to allow recording of the potential over the duct wall at the hilus. Two experiments were made. In neither of them did injection of saline or saliva, causing a larger volume flow through the main duct than that resulting from maximal stimulation of the secretory nerves, change the potential over the duct wall. In another type of experiment the potential over the duct wall resulting from stimulation of the secretory nerves was measured during obstruction of the main duct so that the saliva could not escape. Nevertheless a potential difference was established of about the same magnitude as found when the saliva was allowed to escape. The return of the potential after the end of stimulation was, however, much slower than normally and often incomplete.

Comment. The observation that the chorda electrogram of the submaxillary gland is very variable is in agreement with the findings in the dog made by VAN HARREVELD (1930 a). In experi-

ments on the cat LANGENSKIÖLD observed a far more regular electrogram of the type shown in Fig. 1. Since this type of chorda electrogram can be produced after previous stimulation of the sympathetic nerve the possibility may be considered that in LANGENSKIÖLD's experiments on decerebrate animals an outflow of adrenaline from the suprarenal glands tended to regularize the electrogram. VAN HARREVELD pointed out that the variability of the sign of the chorda electrogram throws doubt on the classical conception that hilus-positivity results on secretion of salt and water whereas hilus-negativity appears on activity in cells giving a viscid saliva. The present experiments support this critical view and the finding that a potential is established over the duct wall with a time course similar to that of the slow phase of the electrogram suggests that a reinterpretation of the salivary gland electrogram is necessary.

2. Microelectrode recordings from the submaxillary gland.

When a microelectrode is inserted into a salivary gland, a sudden shift of the potential may be recorded. It is assumed that these potentials are recorded from single cells in the salivary gland; the potential jump results from a minute movement of the microelectrode, and insertion for a further 5—15 μ usually brings about a new potential jump. With stimulation of the secretory nerves, this potential may shift characteristically. Three different kinds of response have been identified and in the following description and discussion they will be referred to as microelectrode response type I, II and III.

a) *Microelectrode response type I.* This response is the one found most frequently. It is illustrated in Figs. 8—12. Fig. 8 demonstrates the sudden negativity on insertion of the electrode into the surface layer of the gland. Under conditions allowing observation of single acini, the response has been observed on entering one of them. The mean value of the membrane potential (100 successive measurements) is 22 mV with 15 mV as the minimum and 35 as the maximum. On stimulation of the chorda with single shocks the result is, after a latency of 0.2—0.4 sec., a temporary increase of the internal negativity. It may amount to 20 mV and lasts for about 1 sec. In a few glands no potential change resulted from single shocks, but potentials with the same time course were found if a short train of impulses was given to the chorda. If the chorda is stimulated with single

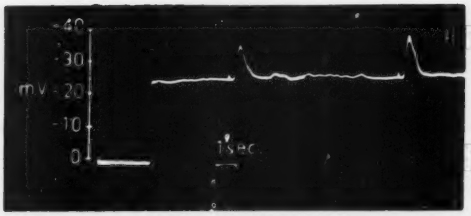

Fig. 8. Potential jump on insertion of microelectrode into an acinus. Increasing internal negativity with single shocks to the chorda.

shocks at 10 sec. interval, the response may be kept for a considerable time (in one case more than 30 minutes) with little change in the resting membrane potential or in the secretory potential.

If the chorda is stimulated repetitively, there is in the great majority of observations a jump of the potential and the response is lost. This is understandable in view of the circulatory changes in the gland and the presence of contractile cells. Usually, the potential reaches a maximum before the jump occurs. In most experiments the potential change appears after a latency ranging from 0.2—0.3 sec., but in some gland it may amount to 0.6 sec. The hyperpolarization usually amounts to 25—35 mV but values up to 40 mV have been encountered. During activity the membrane potential of these cells thus may increase to more than 60 mV. The rate of hyperpolarization is about 150 mV/sec., with variations in different cells from 105—190 mV/sec. in a typical experiment. Whenever the secretory potentials have been recorded fully they have shown the characteristics seen in Fig. 9. It reaches initially a maximum, from which it declines only slightly, to remain on a plateau as long as stimulation lasts. Less than one sec. after the end of stimulation, the secretory potential starts to decline, and after 2—5 sec. the membrane potential has resumed its resting value. Both the latency and the rate of establishment correspond closely to those found for the initial, fast hilus-positivity of the external chorda electrogram. Furthermore, when the latency of the external electrogram in some glands was exceptionally long the same was found for the hyperpolarization measured with internal electrodes.

Fig. 9 also shows that stimulation of the sympathetic nerve results in an increased internal negativity, the latency and initial time course being about the same as in the early phase of the

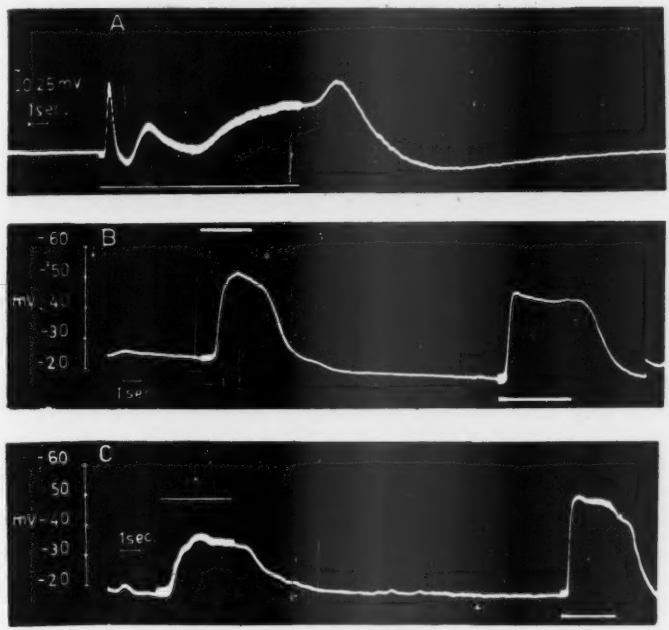

Fig. 9. A. Chorda electrogram recorded between one electrode on the hilus and another on the free surface of the gland. B and C from the same gland as A, show microelectrode response type I with increasing internal negativity on both chorda and sympathetic stimulation. See text.

external sympathetic electrogram. To obtain this response, repetitive stimulation is necessary; there is never an increased membrane potential as the result of a single shock to the cervical sympathetic nerve. This sympathetic secretory potential appears after a longer latency and with a slower rate of rise than the corresponding chorda potential, but it may as in record B in Fig. 6, be of the same magnitude. However, it is often considerably smaller, as shown in record C of the same Figure. In other experiments, when only a small volume flow of saliva resulted from stimulation of the sympathetic nerve, the secretory potentials were still smaller or even absent.

In cases in which the sympathetic secretory potentials are small, stimulation of the chorda during stimulation of the sympathetic nerve increases the membrane potential to the level that

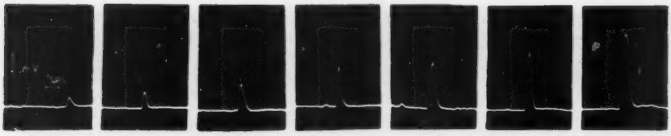

Fig. 10. Response type I showing increasing response with increasing strength of single shock stimulation to chorda. Supramaximal stimulation in record to the right.

could have been expected had only the chorda been stimulated. Stimulation of the sympathetic nerve during stimulation of the chorda does not change the membrane potential.

It is noteworthy that sympathetic and parasympathetic stimulation can activate the same element, as indicated by the absence of any jump in the potential (Fig. 9, record B, potential jump only after the end of chorda stimulation). The degree of common innervation is difficult to examine by the use of tetanic stimulation, since the element is usually lost, but with the use of single shocks to the chorda, followed by repetitive stimulation of the sympathetic nerves, it was ascertained that the cells giving the response can be activated both by sympathetic and parasympathetic stimulation.

The potential resulting from a single shock to the chorda has been studied as a function of stimulus strength. To the right in Fig. 10 is seen the potential resulting from a maximal single shock to the chorda, and to the left is seen how this potential decreases with a progressively weaker stimulus. With the assumption mentioned in the introduction that the recording is from a single cell, this indicates a considerable convergence to one gland cell. Sometimes it has been possible to demonstrate a stepwise increase in the potential with increase of stimulus strength. In Fig. 11, the stimuli have successive numbers; the stimulus strength was slightly increased between 1 and 2 and also between 10 and 11. Response 1 was all or none obtained with just supraliminal stimulus strength. A slight increase of the stimulus caused potentials of three different sizes to appear. There are no intermediate sizes and the picture suggests that the steps in size signify the potential change resulting from impulses in single neurons. It should be remembered that the fibres activated in the chorda are presynaptic to those which actually activate the cells; hence we should expect a greater variation in the number of activated final neurons than if these had been activated directly. Experi-

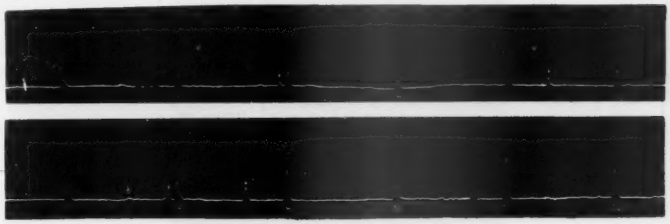

Fig. 11. Response type I. Quantal appearance of potentials on submaximal stimulation of the chorda. Numbers indicate successive single shock stimuli to the chorda. Slight increase in stimulus strength between 1 and 2 and between 10 and 11. Response marked 11 about half maximal. At arrows, potential changes appearing without any stimulation of the chorda.

ments of the type illustrated in Fig. 11 suggest that the gland cell is innervated by at least 5—10 neurons.

In Fig. 11 are shown, marked with arrows, potential changes appearing without any stimulation of the nerve. They are, though smaller, also seen in Fig. 8. They have usually about the same time course as the potentials resulting from a single shock to the chorda, and may sometimes be as large as 10 mV. They are not found regularly, and the mechanism by which they are established is not clear.

The relation of the secretory potential to the secretion of the gland is evident. In such cases in which there is a diminished secretion from the gland after a period of anoxia, or after atropine, the secretory potentials decrease in parallel to the secretion. In this connexion it should be recalled that the initial hilus-positive phase of the electrogram is abolished with secretion by atropine (LANGENSKIÖLD 1941).

Experiments indicate that this potential change resulting on secretion is over the membrane of the cell. An alternative possibility would be an intracellular gradient, but the response resulting from a single shock to the chorda is about the same when the tip of the electrode is just beneath the membrane as when it is gradually pushed 10 μ down.

It is shown in Fig. 12 that acetylcholine, adrenaline or pilocarpine injected into a branch of the external carotid artery increases the internal negativity in the same way as stimulation of the secretory nerves. Only on injection of adrenaline it was possible to maintain the response to obtain the full course of the potential change; with acetylcholine or pilocarpine, a potential

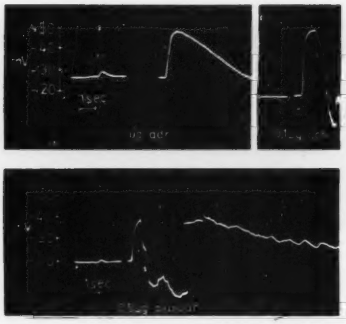

Fig. 12. Microelectrode response type I. Effect of acetylcholine, adrenaline and pilocarpine on membrane potential. Interruption of beam denotes injection of 0.1 ml solution in branch of external carotid artery.

jump followed the initial part of the secretory potential. The external current flow recorded between one electrode on the hilus and another on the free surface of the gland was also studied after injection of acetylcholine and adrenaline. It proved to be much the same as after chorda or sympathetic stimulation, respectively; this also applied to the potential recorded over the duct wall.

Comment. The response described must originate from gland cells, since it was obtained when under visual control the tip had entered an acinus. The secretory potential measured is a change of potential over the outer membrane. Attempts to measure the potential from the lumen of the acini were not successful, and consequently we have no direct knowledge of potentials at rest and during activity over the inner membrane. However, the possibility remains that some information might be gained from the direction of external current flow. In view of the fact that the external electrogram and type I microelectrode response have the same initial time course and also vary in parallel, it is suggested that they originate from the same gland cells. It was assumed by RABL (1922) and by GAYDA (1924) that the saliva in the ducts constitutes a low resistance path for the current generated by the gland cells. An external current flow might be expected when there is a potential difference between the outside medium and the lumen of the acinus, and directed toward the hilus if the lumen were negative to the outside medium. Now if there was no potential change over the inner membrane with secretion the potential change actually occurring over the outer membrane

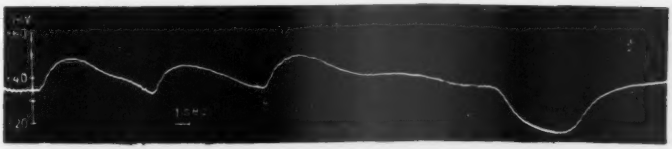

Fig. 13. Microelectrode response type II. Longlasting increasing internal negativity on single shock stimulation of the chorda, and decreasing internal negativity on sympathetic stimulation.

must make the lumen of the acini as well as the cell interior negative to the external medium, with an initial hilus-negativity in the external electrogram as the expected result. Therefore the fact that the external electrogram initially is hilus-positive suggests that at secretion a potential change occurs also over the inner membrane of the cell, and with the sign that the lumen becomes more positive to the cell interior. To account for the hilus-positive external electrogram it is, however, not necessary to assume, that the lumen actually must become positive to the external medium. The gland and duct cells, which make out the core conductor, most likely consists of two high resistance membranes and purely physical conditions may favour current flow from one of them, in this case may be from the inner.

b) *Microelectrode response type II.* The second type of microelectrode response has also been found in the surface layer of the gland, but has actually never been obtained under conditions in which the microelectrode entered an acinus under visual control. The mean value of the resting membrane potential is 32 mV. The potential changes occurring on stimulation of the secretory nerves are seen in Fig. 13. On single shocks to the chorda, an increased internal negativity appears after a latency of 0.4 sec., and with a rate of rise of about 15 mV/sec. The hyperpolarization amounts to 15—20 mV and is, in particular, characterized by a very long duration, *i. e.* 7—10 sec. Repetitive stimulation of the sympathetic nerve results in decreased internal negativity, as also appears in Fig. 13. Thus, in this case, chorda and sympathetic stimulation have reciprocal effects. It was not possible to obtain the secretory response to repetitive stimulation of the chorda, since this procedure invariably caused a jump of potential and loss of response. This, as well as the fact that the response is easily lost even without repetitive stimulation, gives the impression that the microelectrode records from a smaller unit than in the case of

Fig. 14. Microelectrode response type III. Longlasting decreasing negativity on sympathetic stimulation.

type I, but it is realized that other factors than the size of the cells may be responsible. In the submaxillary gland, no potentials were identified which, on single chorda shocks, give an increased internal negativity with a duration intermediate to those typical of response I and II. Moreover in all cases in which the characteristic slow potential was obtained on chorda stimulation, sympathetic stimulation caused depolarization. This type II response was encountered in all the experiments (more than 30). In some cases only a few responses of this type were found, against hundreds of type I, but in some experiments as many as 1/4 of the responses were of type II. It is noteworthy that, if repetitive sympathetic stimulation is administered during the hyperpolarization caused by chorda stimulation, a depolarization results. Similarly, if a single shock to the chorda is given during a depolarization caused by sympathetic activation, a decrease of the depolarization occurs.

All the microelectrode responses described in this paper were also observed with microelectrodes filled with, 0.15 M KCl and 0.15 M NaCl. When either of these electrodes was used microelectrode response type II differed quantitatively from that recorded when the electrodes were filled with 3 M KCl. The hyperpolarization was consistently much larger, often being about 50 mV.

Comment. The type II response has also been found in the actual surface layer of the gland. In the surface layer there are in addition to gland cells connective tissue cells, myo-epithelial cells, ganglion cells and blood vessels. Since, however, it is known that two distinct types of gland cells exist, the suggestion is put forward that also response type II originates from gland cells. The question then arises whether we have any possibility of determining whether this type II response has any equivalent in the external electrogram which may allow interpretation of the mechanism of

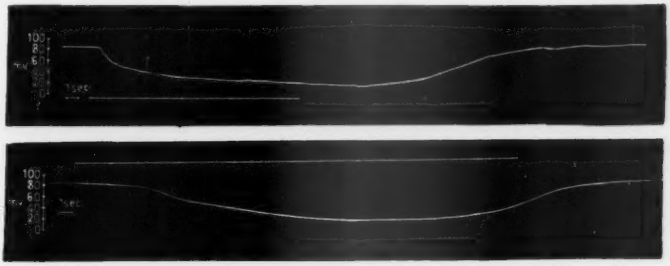

Fig. 15. Microelectrode response type III. Time course of potential change on longlasting repetitive stimulation of the chorda (upper record) and of the sympathetic nerve (lower record).

establishment. There is, however, some evidence that this response does not contribute to the external electrogram. LANGENSKIÖLD demonstrated that a period of sympathetic stimulation during tetanic stimulation of the chorda does not change the chorda electrogram (cf. Fig. 6). Since the potentials recorded with internal electrodes do superimpose we might expect a change of the chorda electrogram if the response type II did at all contribute to the recorded external current flow.

c) *Microelectrode response type III.* When a microelectrode is inserted deeper into the gland, another response in addition to the two already described may be found. It is characterized by a potential jump of about 80 mV, with internal negativity in this case as well. On stimulation of either of the secretory nerves a depolarization results, as shown in Figs. 14—16. The effect of sympathetic stimulation is demonstrated in Fig. 14. It obviously has a time course similar to that of the slow phase of the external electrogram and the potential recorded with a catheter inserted into the duct. The latency, especially on sympathetic stimulation, is not constant as appears in the record in Figs. 14 and 15. During longlasting sympathetic stimulation, the depolarization passes through a maximum (Fig. 15), as does the slow hilus positive phase of the external electrogram and the potential recorded from the duct. In addition, the depolarization on chorda stimulation persists or even continues to increase, for some time after the end of repetitive stimulation. The depolarization is marked and characteristically, the membrane potential in Fig. 15 is reduced to about 20 mV. The record in Fig. 16 demonstrates

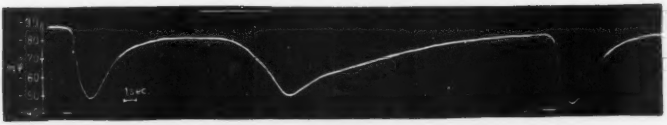

Fig. 16. Microelectrode response type III. Record showing reversible effects of chorda and sympathetic stimulation in the same unit.

depolarization by chorda and sympathetic stimulation in the same element. Even when using only brief tetanic stimulation, it was not often possible to obtain this, because of loss of the unit. The common effect of sympathetic and parasympathetic stimulation is, however, apparent from the finding that when a large membrane potential was recorded depolarization invariably resulted with stimulation of either nerve.

Comment. Two facts suggest that the type III microelectrode response originates from duct cells. The time course is the same as that of the potential recorded from the duct by a catheter and it has been found only in the depth of the gland.

The ducts of the submaxillary gland contain three types of epithelial cells. In the short isthmus (intercalated portion) the epithelium is of a low cuboid type, succeeded by the striated tubuli with striated columnar cells and, finally, the larger ducts with a columnar epithelium without striation. The cells of the striated tubuli greatly resemble those of the proximal convolutions of the kidney. The striation is believed to be caused by parallel rows of mitochondria. The anatomy of the striated tubuli has been studied in the rabbit, dog, horse, and man (MERKEL 1883, ZIMMERMAN 1927) but, unfortunately, I have been unable to find any detailed description of the anatomy in the cat. However, in the animals listed above the submaxillary gland has a very well developed system of striated tubuli.

Judging by the histologic features of the ducts one might expect a transferring function of the striated tubuli (MERKEL 1883). It is probable that the type III response is elicited from the cells of the striated tubuli, the potential change on activity being the electrical sign of ionic transfer.

The microelectrode presumably measures the potential over the outer membrane. If a potential change at activity occurred only over the outer membrane, we would expect the lumen of the duct to become positive to the external medium. However, on recording

with a catheter from the duct interior we found a potential change with the same time course, but in the negative direction. This can be explained if on activation, a potential change occurred over the inner membrane as well, with the sign to make the lumen negative to the cell interior.

For a discussion of a possible ionic basis of these potentials, it is of interest that the literature contains some informations about the function of the striated tubuli. GREGERSEN and INGALLS (1931) demonstrated that the concentration of sodium in saliva increases with increasing volume flow, whereas the concentration of potassium is independent of the volume flow. THAYSEN, THORN and SCHWARTZ (1954) confirmed this observation, and SCHWARTZ, THAYSEN and DOLE (1953) demonstrated that in sweat as well, the concentration of sodium but not that of potassium ions rises with increasing rate of secretion (THAYSEN and THORN, 1954). These authors suggested that there is a reabsorption of sodium in the sweat glands and in the salivary gland examined, but not in the lachrymal gland. It is known that there are striated tubuli in salivary glands and sweat glands, but not in the lachrymal glands. It therefore seems likely that the cells of the striated tubuli have a reabsorptive function. Let it now be assumed that the observed and postulated potential changes are caused by ionic fluxes with the same direction as the osmotic work done by these duct cells. It then follows that from the lumen over the inner membrane there is a faster transfer of cations than of anions, and from the cell interior over the outer membrane a faster transfer of anions than of cations.

The similarities between, on one hand, the time course of the duct potential and that of the type III microelectrode response and, on the other hand, between the slow phase of the external sympathetic electrogram and the chorda electrogram, are striking. It was already suggested in the first section of this paper that the duct cells contribute to the external electrogram. The following question then arises. Why does the potential generated by the duct cells always have the same polarity, but the slow phase of the external electrogram may be hilus-positive, hilus-negative or altogether absent, whereas the initial phase of the electrogram invariably has the same sign? The reason may be that two alternative loops exist for the current generated by the duct cells. From the basal membrane, the current may pass towards the hilus or towards the acinus, back through the lumen and the cell.

With the former alternative, external electrodes would record hilus-negativity, with the latter hilus-positivity. The main direction of current flow would thus be determined by the relation between the resistances of these alternative loops. There are certainly other possibilities to account for the observed variability of the sign of the slow phase of the external electrogram, the scheme outlined above may at least serve to emphasize the impression that the sign of the slow phase of the external electrogram does not necessarily give information of the sign of the potentials generated over the duct cell membranes.

Discussion.

The attempt to measure the potentials of the gland cells have met with partial success, in so far as only the resting and active potential over the outer membrane have been measured. When the gland cell is activated this membrane is hyperpolarized, most likely due to a faster entry of anions than of cations (type I response). The suggestion that the inner membrane is hyperpolarized when the cell is activated is based on the direction of the external current flow, and this hyperpolarization may be due to a faster outflux of cations than of anions to the lumen of the acinus.

It is of interest to analyse the mechanism of establishment of these potentials since it is possible that they are the electric sign of a transport, representing the osmotic work of secretion. However, the submaxillary gland is not a suitable preparation for quantitative work; we cannot record from the lumen of the acini, it contains two types of gland cells and we do not know the composition and amount of "primary saliva". In all these respects the sublingual gland has proved more favourable and further work on the significance of the secretory potentials has been carried out with this gland.

It would be of interest to know, whether type I response originates from mucous or from demilune cells; unfortunately this cannot be determined from the present experiments. An attempt was made at a further study of this question by experiments on the sublingual gland, which produces a highly viscid saliva. A microelectrode response from gland cells was found in this gland, but the time course of the potential change differed charac-

teristically from both responses I and II in the submaxillary gland. Consequently, it is not possible to apply findings from one gland to another. A suggestion that the type I response originates from cells producing a watery saliva (LUNDBERG, 1954) was made before this was realized.

The function of the striated tubuli has been discussed in detail above in the aforegoing. In connection with the conclusion that response type III, as well as the slow phase of the external electrogram, are elicited from the cells of the striated tubuli, it is noteworthy that the external electrogram of the sublingual gland does not have a slow phase, and that no type III microelectrode response was ever encountered in this gland (unpublished observation). Histologically, this gland is characterized by the lack of regular striated tubuli, only a few islands of striated cells being present (MERKEL, 1883, JÄRVI, 1939).

The microelectrode recordings are of interest in relation to problems concerning the innervation of gland cells (cf. BABKIN, 1944, HILLARP, 1948, EMMELIN, 1955). An interesting feature is that in all three types of response studied, stimulation of the chorda and of the sympathetic nerve activate the same unit. It is likely, although not certain, that the response from the duct cells is due to active innervation of these cells. However, with regard to responses I and II, the results indicate that sympathetic and parasympathetic nerve fibres converge to the same cell, and also that there is a considerable convergence of terminals from different parasympathetic neurons to one gland cell. Of interest is the finding that in the type II response, sympathetic and parasympathetic stimulation produce potential shifts of opposite sign. Observations on the varying composition of saliva on sympathetic and parasympathetic stimulation (KOMAROV and STAVRAKY, 1940), as well as the findings of RAWLINSON (1933), have been taken to indicate that some cells have sympathetic innervation only, and other cells parasympathetic only (cf. BABKIN, 1944). The present experiments suggest the possibility that such a differential effect may be caused by the activation of different secretory processes in one type of gland cells.

Summary.

The electrophysiology of the submaxillary gland of the cat has been studied with external and intracellular electrodes.

The external chorda electrogram led off between the hilus and the free surface of the gland is not constant except for the initial, fast hilus-positivity. After this initial phase, a slow phase appears, which may be hilus-negative or, as in the sympathetic electrogram, hilus-positive.

When recording over the duct wall inside the hilus, stimulation of the chorda or of the sympathetic nerve results in negativity of the duct interior. The time course of this potential change resembles that of the slow phase of the respective external electrogram.

In experiments with microelectrodes, three types of responses were identified, the differentiation depending on the magnitude of the membrane potential, as well as on the sign and time course of the potential change occurring on stimulation of the secretory nerves. It is assumed that these potentials are recorded from single cells.

Response type I, originating from gland cells, has a resting potential over the outer membrane of about 22 mV (cell interior negative). Stimulation of either of the secretory nerves increases the potential over the outer membrane to 45—60 mV, the latency and time course of this increase being similar to that of the initial phase of the external electrogram. It is suggested that the direction of current flow signifies that the lumen of the acinus is becoming positive to the cell interior.

Response type II, with a resting membrane potential of about 33 mV, is less often found. Single shocks to the chorda increase the internal negativity for 7—10 sec., whereas repetitive stimulation of the sympathetic nerve results in decreased internal negativity. There is no indication that these potentials have any equivalents in the external electrogram.

Response type III, with a resting internal negativity of 80 mV, is found only in the depth of the gland. Stimulation of either of the secretory nerves decreases this internal negativity, the time course being similar to that of the potential change recorded over the wall of the duct. It is suggested that this response originates from the cells of the striated tubuli, and that in addition to the potential change over the outer membrane directly measured, there is at activity also a potential change over the inner membrane with the sign that the lumen becomes negative to the cell interior.

The significance of these findings with respect to the mechanism

of secretion and of problems concerning the innervation of gland cells is discussed.

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Oxygen and Carbon Dioxide Tension in the Umbilical Vessels of the Human Fetus in Prolonged Asphyxia.

By

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Received 15 April 1955.

The oxygen saturation in the umbilical vein of deeply asphyxiated newborn infants is extremely low (EASTMAN 1932). However, no similar investigations have been performed in human fetuses before the fifth month of gestation, this being of special interest as the supply of oxygen of the fetuses after birth is entirely dependent on diffusion respiration of the skin. The oral cavity and the bronchial tree are filled with liquid. Primitive respiratory movements, so called gasps, are not capable to expand the athelectatic lungs.

The present paper is a continuation of previous investigations of the asphyxiated fetus (ENHÖRNING and WESTIN 1951, 1952, 1954 and 1955.)

Material and Method.

Ten human fetuses ranging in length from 15—27 cm were obtained from legally induced abortions. The operations were performed in ether anaesthesia and oxygen was administered to the mother.

The fetuses were studied at a room temperature of 23° C and relative humidity of 35 %. The rectal temperature of some fetuses was recorded continuously and was found to decrease rapidly. A temperature of 30° C was reached after about 15 minutes and 25° C after 60—75 minutes. One or two blood samples of 2 ml were drawn from the umbilical vein and one artery after different lapse of time after birth.

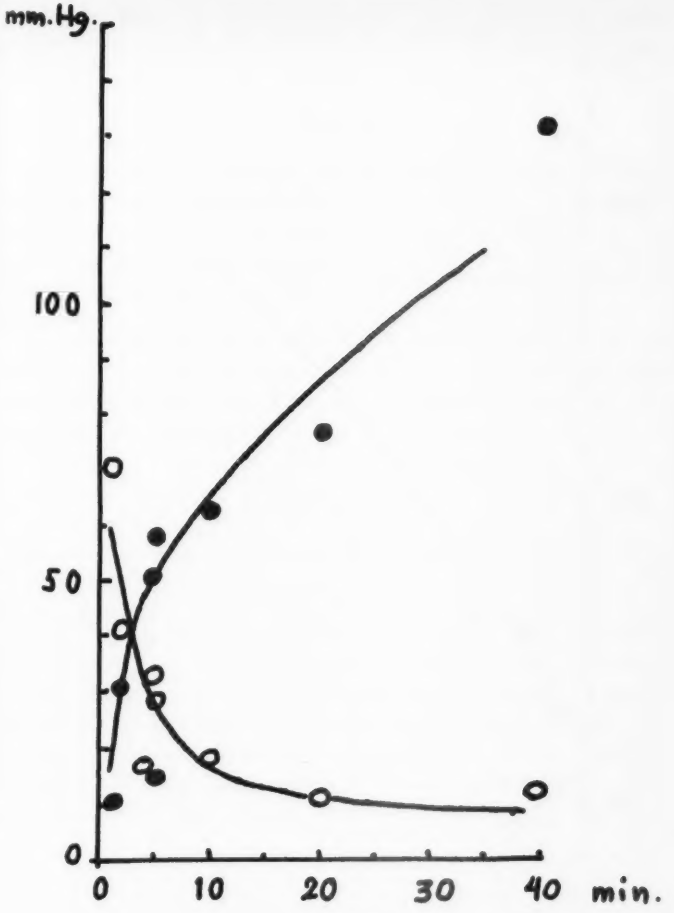

Fig. I. Umbilical vein.
p O_2 = ○
p CO_2 = ●

The umbilical cord was not clamped. Glass syringes moistened with heparin were used. The blood samples were taken to examination within a few minutes.

The microvolumetric determinations of oxygen and carbon dioxide tensions in the blood were performed according to RILEY, PROEMMEL and FRANKE (1945) with a slight modification described by BJÖRK

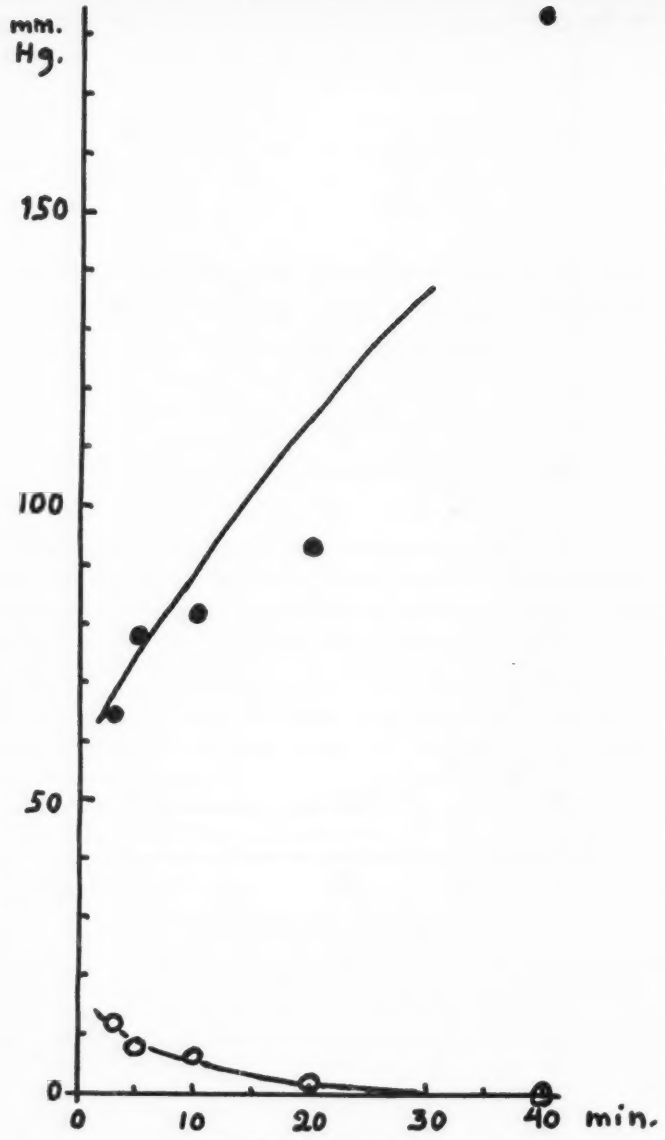

Fig. II. Umbilical artery.
p O_2 = ○
p CO_2 = ●

and HILTY (1954). The ROUGHTON-SCHOLANDER (1943) type of syringe analyzer was used. Double determinations showed a method error of 2 per cent.

Results.

The results of oxygen and carbon dioxide determinations (p O_2 and p CO_2) of the umbilical *vein* are collected in fig. I. The highest initial value for p O_2 was 70 mm mercury, thereafter there appeared to be a steady decrease with increasing lapse of time. The slope of p CO_2 indicated an inverse ratio tending to extremely high values.

Corresponding results of p O_2 and p CO_2 of the umbilical *arteries* are indicated in fig. II. In comparison with the results obtained from the umbilical vein there is a significant decrease in p O_2. respectively increase of p CO_2.

Discussion.

Metabolism is dependent on body temperature. It therefore seems possible that the slopes of oxygen tension of the blood are related to the decrease of body temperature. On the other hand it is evident that diffusion respiration of the skin from air of atmospheric pressure can not maintain the initial oxygen tension of the blood. As no lung ventilation occurs excessive amounts of carbon dioxide are accumulated in the blood. Finally the withdrawal of 4—8 ml of blood from the little blood volume of fetuses of this size may cause some errors in the results.

Summary.

In ten human fetuses ranging in length from 15—27 cm oxygen and carbon dioxide tensions were determined according to a method described by BJÖRK and HILTY (1954). In the umbilical *vein* there was a decrease of oxygen tension with increasing elapse of time after birth. The slope of p CO_2 indicated an inverse ratio. In comparison with the results obtained from the umbilical vein there is a significant decrease of p O_2, respectively increase of p CO_2 in the umbilical *arteries*. The relation of body temperature, diffusion respiration of the skin, absence of lung ventilation and diminished blood volume to the results is discussed.

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A Combination of Rhodanese and Ethanethiosulfonate as an Antidote in Experimental Cyanide Poisoning.

By

CARL-JOHAN CLEMEDSON, HOLTER I:SON HULTMAN and BO SÖRBO.

Received 4 May 1955.

Recently a combination of thiosulfate and rhodanese was reported by CLEMEDSON, HULTMAN and SÖRBO (1954) to be a very active antidote in the treatment of cyanide poisoning. Rhodanese is an enzyme which in the presence of thiosulfate transforms cyanide (hydrocyanic acid) into the much less toxic thiocyanate ion (LANG 1933). In this reaction thiosulfate can be replaced as a sulfur donor by thiosulfonates which are more active than thiosulfate in this respect (SÖRBO 1953 a). Consequently it could be expected that a combination of rhodanese and a thiosulfonate should be a very effective antidote and this has been verified in the present investigation which was carried out with ethanethiosulfonate as the sulfur donor. In vitro experiments have shown that this compound is about ten times more active than thiosulfate in the rhodanese reaction (SÖRBO 1953 b).

Methods.

Sodium ethanethiosulfonate and crystalline rhodanese were prepared as described previously (SÖRBO 1953 b and c).

The prophylactic effect of sodium ethanethiosulfonate alone and combined with rhodanese was determined in 50 rabbits according to the technique described in the preceding publication (CLEMEDSON et

coll. 1954). Rhodanese, corresponding to 750 RU/kg body weight and ethanethiosulfonate, corresponding to a 4-fold molar equivalent of the injected cyanide were first injected in one of the marginal ear veins, immediately followed by hydrocyanic acid in another ear vein. The concentration of the hydrocyanic acid injected (KCN in saline solution, neutralized with HCl) was 1 mg HCN/ml in the experiments with ethanethiosulfonate alone and 10 mg/ml in the experiments with rhodanese and ethanethiosulfonate. The time for injection of the poison was exactly 30 seconds. The LD_{50} for HCN was estimated by gross inspection of the data obtained.

The therapeutic effect of rhodanese and ethanethiosulfonate was demonstrated in rabbits given a rapid injection of cyanide prior to the injection of the antidote. The latter was injected as soon as the corneal reflex disappeared (about 1 minute after the injection of the poison was finished) and as respiratory paralysis occurred at the same time, the animals had to be given artificial respiration in order to survive. The therapeutic effect was also demonstrated in rabbits which were given a slow injection of cyanide (rate of injection 0.2 mg HCN/min.) until the respiration rate was slowed down to 6/min. The rabbits were now moribund. Rhodanese (750 RU/kg) and ethanethiosulfonate (4-fold molar equivalent of the injected cyanide) was then injected and the time noted, when the animals had recovered and could walk freely again.

Results.

The prophylactic effect of sodium ethanethiosulfonate alone was investigated in a series of 25 rabbits as shown in table I. The LD_{50} was about 0.8 mg HCN/kg body weight and did not significantly differ from the value 0.7 mg HCN/kg body weight obtained in animals without any antidote (CLEMEDSON, HULTMAN and SÖRBO 1954). The effect of a combination of ethanethiosulfonate and rhodanese is shown in table II. The LD_{50} was now found to be about 13 mg HCN/kg and a 15 to 20-fold increase of the LD_{50} was thus obtained.

The therapeutic effect of rhodanese and ethanethiosulfonate was demonstrated in 5 rabbits, each receiving 1.5 mg HCN/kg (2 LD_{50} or 1 LD_{100}) in a rapid intravenous injection. Of these animals 4 recovered, and in the case of the dead animal the heart probably stopped before the injection of the antidote was started. The therapeutic effect was also demonstrated in 2 animals given a slow injection of cyanide (see Methods). Both had received 2.5 mg HCN/kg body weight when the respiration stopped but they nevertheless recovered and were walking around 13 and 16 minutes respectively after the antidote was given.

Table I.

Toxicity of intravenously injected cyanide after previous injection of ethanethiosulfonate.

Ethanethiosulfonate dose = 4-fold molar equivalent of cyanide.

Dose mg HCN/kg body weight	Results	
	living	dead
0.6	4	1
0.8	3	2
1.0	1	4
1.2	2	3
1.4	0	5
LD_{50} = about 0.8 mg HCN/kg body weight		

Table II.

Toxicity of intravenously injected cyanide after previous injection of ethanethiosulfonate and rhodanese.

Ethanethiosulfonate dose = 4-fold molar equivalent of cyanide, rhodanese dose = 750 RU/kg body weight.

Dose mg HCN/kg body weight	Results	
	living	dead
9.0	5	0
11.5	3	2
13.0	2	3
15.0	2	3
18.0	0	5
LD_{50} = about 13 mg HCN/kg body weight		

Discussion.

Since rhodanese is already present in large amounts in the body of vertebrates the antidote effect of intravenously injected rhodanese may appear somewhat unexpected. The enzyme occurs primarily in the liver and kidneys (LANG 1933, HIMWICH and SAUNDERS 1948) but lacks in the blood. As it is localized inside the cell it was pointed out by HIMWICH and SAUNDERS (1948) that the low permeability of thiosulfate through the cell mem-

brane may explain the limited antidote effect of this compound. The same phenomenon is presumably also valid for the thiosulfonates. If, however, rhodanese is injected in combination with the sulfur donor in the blood stream, a detoxification of cyanide (hydrocyanic acid) outside the cell will be the result, as cyanide can permeate through the cell membranes.

In the preceding paper (CLEMEDSON et coll. 1954) thiosulfate alone was found to be without antidote effect, but it was pointed out that these results were obtained at a dose level of thiosulfate of about 10 mg/kg, whereas CHEN et al. (1952) recommend a dose of 200 mg/kg in the treatment of human cases of cyanide poisoning. We have tested the antidote effect of thiosulfate in rabbits at this higher dose level (unpublished) and obtained a 2-fold increase of the LD_{50} for rapidly injected HCN. It is possible, that also ethanethiosulfonate will show a definite antidote effect at higher dose levels, but enough material was not available to test this possibility.

Of interest in this connection is the toxicity of sodium ethanethiosulfonate. Preliminary experiments on mice (unpublished) have shown that the compound is practically nontoxic and that the animals tolerate such high dose levels as 500 mg/kg very well. The metabolic fate of injected ethanethiosulfonate is unknown but will be investigated in separate experiments. No experiments on the antidote effect of rhodanese alone have been carried out, but no significant effect can be expected, since thiosulfate, the natural sulfur donor, only occurs in trace amounts in the body (GAST, ARAI and ALDRICH, 1952).

Summary.

The LD_{50} for rapidly intravenously injected HCN was increased about 15 to 20 times by a previous intravenous injection of rhodanese and ethanethiosulfonate. The therapeutic effect in cyanide poisoning of this combination was also demonstrated.

The ethanethiosulfonate used in the present investigation was synthesized by Dr. L.-E. TAMMÉLIN to whom the authors wish to express their sincere gratitude.

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A Study on the Uptake of Injected Radioiron in the Mouse Kidney.

By

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The kidney is known to accumulate rather large amounts of radioiron. The distribution of radioiron among the various structures of the kidney is, however, known only to a fairly small degree. We therefore undertook the following study on the uptake of injected radioiron in the mouse kidney, partly using scintillation counting, partly autoradiography.

Experimental.

Nine mice were given 1.0 μC of radioiron (Fe^{59}) per g of body weight. The radioiron had a specific activity of 65 μC/g and was present in the chemical form of ferric chloride. The injections were given subcutaneously. The mice were killed by bleeding in ether anesthesia, three after six hours, three after two days and three after nine days. One of the kidneys was weighed and counted in a scintillation counter operated with a geometrical efficiency and discriminator setting so that 0.1 μC of Fe^{59} gave 6,800 counts per minute.

The other kidney was fixed in pure methanol, embedded in paraffin and cut in 10 μ thick sections which were mounted on methacrylate slides and deparaffinized. They were then exposed partly on Gevaert Dentus Rapid film, partly on Agfa Printon Rapid film. The exposure time was 65 days. The films were developed in Kodak D 76 for 5 minutes at + 18° and the histological sections were stained with hematoxylin-van Gieson and mounted in Diatex. The films and the sections were then compared under the microscope.

Results.

1. Results of scintillation countings.

The uptake of Fe^{59} in μC per g of kidney is given in table I. There is a tendency of rise with time after injection of radioiron.

Table I.

Uptake of Fe^{59} in μC per gram kidney.

Time after injection	Uptake in individual animals			Mean
6 hours	0.41	0.78	0.26	0.48
2 days	0.56	0.59	0.49	0.55
9 days	0.86	0.80	1.09	0.91

2. Results of autoradiographic studies.

The autoradiographic image of the kidneys underwent considerable changes with time after injection. Some examples are given in fig. 1. The autoradiographic resolution (about 16 μ for Gevaert Dentus Rapid film and about 12 μ for Agfa Printon film) did not permit detailed studies.

The *capsula* of the kidney always contained small but distinct amounts of radioiron, which apparently did not change with time after injection. The *perirenal fat* contained hardly detectable amounts of radioiron.

Blood was seen in some large vessels. In agreement with scintillation countings of whole blood, the autoradiographs showed small amounts at the first and last point of time studied, and very small amounts at the intermediate point of time. The *walls of the large blood vessels* behaved in a manner similar to the renal capsule.

In the cortex, the *glomeruli* contained small or moderate amounts of radioiron. Possible changes with time are difficult to decide because of the presence of cross-radiation from neighbouring tissues. The *proximal convoluted tubules* always contained large amounts of radioiron, which seemed to increase with time after injection. The *loop of Henle* contained large amounts of radioiron at the first time interval studied, but later they decreased to negligible levels. The *distal convoluted tubules* contained probably fairly small amounts of radioiron, but this result is

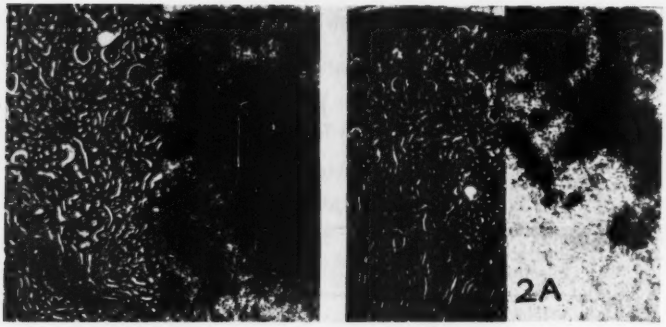

Fig. 1. Magnifications ($\times$ 24) of some representative parts of histological sections (S) and autoradiographs (A, dark parts = radioactivity). L 1S and 1A are taken from animal killed 6 hours after injection and 2S and 2A from an animal killed 9 days after injection of radioiron.

somewhat uncertain for the two earliest points of time studied. The *collecting tubules* in the cortex probably did not contain any considerable amounts of radioactivity.

The *medulla* contained as a rule small amounts of radioiron, somewhat more during the earliest point of time. The radioiron was then probably preferentially localized in the peripheral parts of the marrow corresponding to that part of the loop of Henle which is situated in the medulla. At the two latest time intervals the distribution of radioactivity was essentially uniform and the amounts were slight.

The *urine* in the renal pelvis did not show signs of radioactivity, which may be due to dissolution of radioiron in the histological treatment.

The *renal pelvis* and *upper part of the ureter* contained small amounts of radioiron both in epithelium and in the renal connective tissue.

When considering the autoradiographic pictures as a whole, the most dominating part in the last time interval was the proximal convoluted tubule. At the earlier points of time several structures in the renal cortex took up heavy amounts of radioiron.

Discussion.

The high uptake of radioiron in the kidney, especially in the proximal convoluted tubules is in agreement with the findings of

FINCH et al. (1950). This finding is remarkable and no certain explanation can be given for this phenomenon. Common histochemical tests for iron are negative on kidneys (ANDERSSON, 1950). The possibility that iron is reabsorbed in the tubuli was discussed as early as in 1941 by YUILE, STEINMAN, HAHN and CLARK on the basis of experiments with labelled hemoglobin for injection. From our observations it is impossible to say if the radioiron in the proximal convoluted tubules is reabsorbed iron or not.

The kidney is known to contain fairly large amounts of cytochrome oxidase (ZIEGENHAGEN, AMES and ELVEHJEM, 1947, KRETCHMER and DICKERMAN, 1953). It is not possible to decide if the presence of this or other iron-containing porphyrins can explain our findings or not.

Summary.

Subcutaneously injected $Fe^{59}Cl_3$ in mice accumulates to a fairly large extent in the kidneys. The first few days after injection several structures (except glomeruli) in the cortex contained much radioiron. Nine days after injection the radioiron was mainly localized in proximal convoluted tubules. The marrow contained small amounts of radioiron throughout. The mechanisms underlying the found distribution of radioactive iron are unexplained by the present experiments.

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The Normal Variation of Human Blood Cholinesterase Activity.

By

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The cholinesterase activity of human blood has been studied in considerable detail in order to correlate, if possible, the enzyme activity with various diseases. The results obtained, however, are not easy to interpret because of the marked variation in the cholinesterase activity of normal human subjects. This is especially true for the blood plasma cholinesterase. In addition, the esterase values of one and the same individual are said to vary when estimated over a long period of time. Normal physiological processes have no influence on cholinesterase activity. From this general rule the plasma cholinesterase of women is an exception; this activity is under the influence of female sex hormones and varies accordingly during the menstrual cycle. For details the reader is referred to recent reviews (AUGUSTINSSON 1948, 1950).

There has been a considerable interest in human blood cholinesterase during the last ten years. The reason for this interest is, in the first place, the increasing use of toxic organophosphorus compounds as insecticides and the expected use of such compounds as war gases ("nerve gases"). These compounds are strong inactivators of cholinesterases, a property that is certainly the main reason for their high toxicity. The need for routine analysis of blood cholinesterase activity in clinical laboratories and elsewhere is therefore obvious and a more complete knowledge of the normal blood cholinesterase activity in human subjects and

of its variation is a necessary qualification for an interpretation of the results obtained. Studies of the normal variation of blood cholinesterase activity have been published, but only a few of them are based on material sufficiently large to be of value in a statistical analysis of the results.

The present paper is a report of the results of the determinations of blood cholinesterase activity obtained during the last four years in this laboratory based on 391 blood samples taken from 201 healthy human subjects. The method used for enzyme activity determination will be described in detail. The main difference between the technique used in the present investigation and those reported by other workers is that the determinations have been carried out on whole blood (a drop of blood applied on paper) using specific substrates for the plasma and erythrocyte esterases.

Method.

Principle. The cholinesterase activity is estimated with the WARBURG gasometric technique (AUGUSTINSSON 1948), using 0.05 ml of whole blood as enzyme source applied and dried on filter paper (AUGUSTINSSON and HEIMBÜRGER 1953). Butyrylcholine and acetyl-β-methylcholine ("Mecholyl") are used as specific substrates for plasma and erythrocyte cholinesterase respectively (AUGUSTINSSON and NACHMANSOHN 1949). The reaction is carried out in a bicarbonate buffer solution; acid is produced and an equivalent amount of carbon dioxide is released and measured manometrically. Enzyme activity is expressed in b_{30} values, *i. e.*, amount of CO_2 in μl evolved during 30 minutes, corrections made for nonenzymic hydrolysis of the substrates.

Equipment. A WARBURG apparatus of general type is used. Total capacity of the flasks should be approximately 20 ml with one side bulb and preferably no centre tube. The thermostat temperature is 25° C. For each complete activity determination (duplicate tests carried out with each substrate) 4 manometers with flasks are necessary.

Blood sampling. Whole blood, exactly 0.05 ml, is collected with a blood pipette (heparinized) from a punctured finger. This blood volume is applied on filter paper (Munktell No. 3) and air dried at room temperature (approximately 30 minutes; the samples are carefully dried before they are put away for storing in the refrigerator for later use or put into an envelope to be sent by mail). For each complete analysis 4 blood samples (0.05 ml each applied on separate filter papers) are used. If kept under dry conditions in the refrigerator the samples can be stored for one to two months without any appreciable loss of cholinesterase activity.

Simultaneous with the blood sampling described, blood is taken for hematocrit. Information of the hematocrit value, however, is not always absolutely necessary for evaluating the results (see further "Discussion" below).

Reagents. Buffer solution of pH 7.6 is prepared by mixing the following solutions (distilled water): 100.0 ml of 90 % sodium chloride, 30 ml of 1.26 % sodium bicarbonate, and 2.0 ml of 1.76 % magnesium chloride ($MgCl_2 \cdot 6H_2O$). This mixture is saturated with a gas mixture containing 95 % nitrogen and 5 % carbon dioxide (by volume). Calcium carbonate is formed if the solution is not in equilibrium with at least 5 % CO_2. This buffer has to be renewed each second or third day.

Substrates. Stock solutions of butyrylcholine iodide (BuCh) and acetyl-β-methylcholine iodide (MeCh) are prepared by dissolving the pure compounds in hydrochloric acid of pH 4.0, at which pH these esters are stable for months; the concentrations of the stock solutions are 7.3 % BuCh and 7.15 % MeCh. Immediately before use the stock solutions are diluted with four parts of bicarbonate buffer. The final concentrations of the substrates during activity measurements are (total volume of the reaction mixture, 3.00 ml): 6.47×10^{-3} *M* BuCh and 6.64×10^{-3} *M* MeCh. The spontaneous hydrolysis values are: BuCh, 3; MeCh, 4; these values (expressed in μl CO_2 per 30 minutes) are to be subtracted from the total hydrolysis values obtained (see further below). The spontaneous hydrolysis of the substrates at various pH values and temperatures have recently been investigated by Larsson (1954).

Procedure. One blood spot (corresponding to 0.05 ml of whole blood) is used for each determination. The spot is cut out from the paper, placed in small pieces in the main compartment of the Warburg flask and 2.6 ml of bicarbonate buffer is added. The side bulb is filled with 0.4 ml of the substrate solution. Duplicate runs are carried out with each substrate.

The flasks are connected to manometers and the gas mixture (95 % N_2 + 5 % CO_2) bubbled through. Before the contents of the flasks are mixed temperature equilibrium is attained by shaking in the water thermostat (25°) for about 20 minutes. The first manometer is read immediately before enzyme and substrate are mixed at zero time. At one minute or half a minute intervals the other manometers are read and the contents mixed. Each manometer is read at 5—10 minute intervals continuously for 35—40 minutes. In all determinations a thermobarometer (filled with 3.00 ml water) is used for corrections due to alterations in temperature and atmospheric pressure.

The amount of CO_2 expressed in μl is plotted against time in minutes. The slope of the straight line thus obtained is used as a measure of enzyme activity. The extrapolated 30 minute value ($= a_{30}$) minus the amount of CO_2 evolved during the same period of time by spontaneous (nonenzymic) hydrolysis of the substrates (3 for BuCh and 4 for MeCh) is used as the unit in expressing the cholinesterase activity and symbolised with b_{30}.

Table 1.

Cholinesterase activity determined by the method described in the present paper (example).

Duplicate values noted by (1) and (2).

	Activity expressed as	BuCh (plasma)		MeCh (erythrocytes)	
		(1)	(2)	(1)	(2)
0.05 ml of blood	b_{30}	66.5	66	34	34
	μmoles	2.97	2.95	1.52	1.52
0.01 ml of plasma or erythrocytes	b_{30}	226	228	162	162
Hematocrit 42 %	μmoles	10.1	10.2	7.2	7.2

The b_{30} values are easily converted to μmoles of substrate by using the correction factor 1/22.4, *i. e.*, $b_{30}/22.4$ μmoles of substrate hydrolysed enzymically during 30 minutes.

Example. See Table 1.

Results.

A total of 381 determinations of plasma (butyryl)cholinesterase activity and 391 determinations of erythrocyte acetylcholinesterase activity were carried out on blood samples taken from 201 healthy human individuals, 141 males and 60 females, between 20 and 50 years old. No more than three samples were taken from one individual and the time interval between each sample in these cases was two weeks or more. For 177 samples in the plasma determinations and 205 samples in the erythrocyte determinations the hematocrit values were known.

The distribution and activity diagrams of the plasma butyrylcholinesterase obtained are shown in Figs. 1 and 2 and the diagrams of the erythrocyte acetylcholinesterase in Figs. 3 and 4. The enzyme activity is expressed in b_{30} per 0.05 ml of whole blood (hematocrit values unknown) (Figs. 1 and 3) or in b_{30} per 0.1 ml of plasma/erythrocytes (calculated from the values obtained with whole blood when the hematocrit values were known) (Figs. 2 and 4). The arithmetic mean, the standard deviation and the coefficient of variation (percentage standard deviation) are tabulated in Table 2. The results obtained converted to percentage values are compared with the results reported by other workers using different techniques, in Tables 3 and 4.

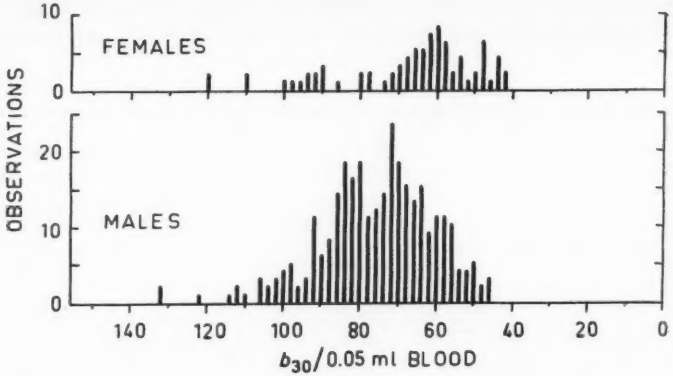

Fig. 1. Distribution and activity diagrams of human plasma (butyryl)cholinesterase obtained with whole blood in 300 determinations on 141 healthy males and in 81 determinations on 60 healthy females. Activity expressed as b_{30} per 0.05 ml of whole blood. Class interval, 2 μl CO_2.

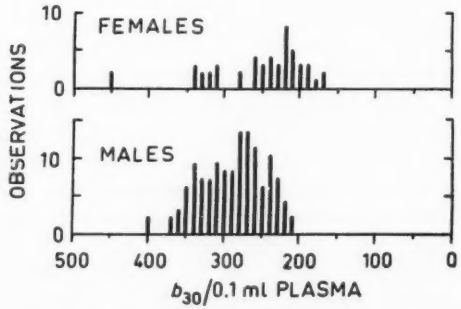

Fig. 2. Distribution and activity diagrams of human plasma (butyryl)cholinesterase obtained with whole blood in 127 determinations on 51 healthy males and in 50 determinations on 31 healthy females. Activity expressed as b_{30} per 0.1 ml plasma (hematocrit values known). Class interval, 10 μl CO_2.

It is seen in Figs. 1 and 2 that the distribution of plasma cholinesterase activity values for males differs from that for females. The difference between the means of the male and female groups is statistically significant. In the female group the variation of the plasma esterase activity during the menstrual cycle has not been considered. The distribution curve in this case is therefore not expected to be "normal".

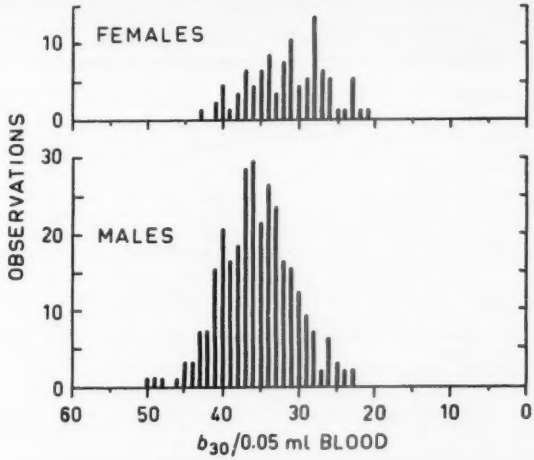

Fig. 3. Distribution and activity diagrams of human erythrocyte acetylcholinesterase obtained with whole blood in 294 determinations on 116 healthy males and in 97 determinations on 47 healthy females. Activity expressed as b_{30} per 0.05 ml of whole blood. Class interval, 1 μl CO_2.

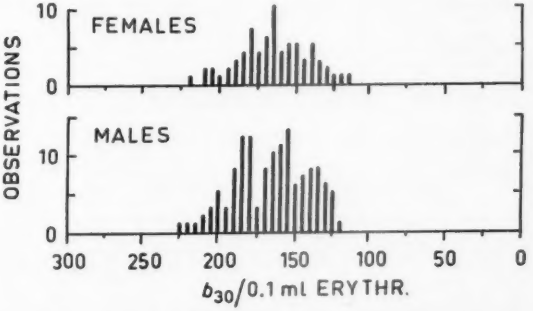

Fig. 4. Distribution and activity diagrams of human erythrocyte acetylcholinesterase obtained with whole blood in 133 determinations on 49 healthy males and in 72 determinations on 29 healthy females. Activity expressed as b_{30} per 0.1 ml erythrocytes (hematocrit values known). Class interval, 5 μl CO_2.

There is no sex differences in the erythrocyte acetylcholinesterase activity (Fig. 4). Such a difference obtained when the activity is expressed in b_{30} per 0.05 ml blood (Fig. 3) is due to the sex difference in the hematocrit values.

Table 2.

Normal blood cholinesterase activity.

Enzyme/Substrate		Plasma BuCh		Erythrocytes MeCh	
Sex		Males	Females	Males	Females
b_{30} per 0.05 ml of whole blood	No. of Subjects	141	60	116	47
	No. of Determinations ...	300	81	294	97
	Mean	75.5	67.2	35.4	31.4
	Standard Deviation	14.7	18.0	4.7	4.9
	Coeff. of Variation	19.4	26.8	13.3	15.6
b_{30} per 0.1 ml of plasma/ erythrocytes	No. of Subjects	51	31	49	29
	No. of Determinations ...	127	50	133	72
	Mean	288.7	252.6	165.4	165.4
	Standard Deviation	42.4	57.9	23.7	22.6
	Coeff. of Variation	14.7	22.9	14.3	13.7

The blood cholinesterase activity of one individual (male) has been determined during a period of 2 years and 3 months and the results are shown in Fig. 5. The erythrocyte esterase activity is constant from week to week. The weekly variation of the plasma cholinesterase activity, on the other hand, is about ± 6 %. A similar observation was recently reported by HEIM (1944).

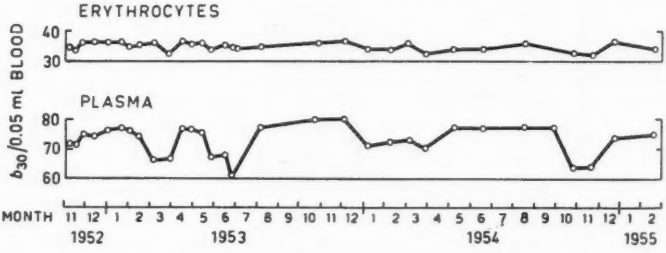

Fig. 5. Normal variation of blood cholinesterase activity of one human male (born 1917) during 2 years and 3 months. The hematocrit value during this period was 44.5 ± 0.5 %.

Discussion.

A great many series of investigations have been published on the blood cholinesterase levels and range of personal variation in healthy adult populations. The results obtained by some observers using different techniques are statistically evaluated

Table 8.

Comparison of the limits of blood plasma (serum) cholinesterase activity of healthy humans determined by various authors.

etylcholine used as substrate, unless otherwise stated. Number of observations are the number individuals (adult humans, unless otherwise stated); when more than one observation was ade on each subject, the total number of observations are inserted in brackets. Range means e smallest and largest observations recalculated from the absolute activity values reported, ith the mean taken as 100. Midrange is the average of the smallest and largest observations ean = 100). In those cases when the standard deviation (used here in calculating the coeffi-ent of variation, in brackets) has not been calculated in the original paper, this value was estimated from the range using Tippett's numbers (a_n).

Authors	Year	Technique	Sex	No. of observations	Range Mean = 100	Midrange	Coeff. of variation
Wahlquist	1935	Titration	Mixed	50	71.7—143	107	19.5
Werebély	1936	Biological[a]	Males	50	31.0—169.0	100	37.8
			Females	50	33 —169	101	35.2
Abdon and Uvnäs	1937	Colorimetric	Males	26	83.8—116	100	10
Hall and Lucas	1937	Titration	Males	19			
			Females	21	(250)[b] 52.8—144.5	99	(21.2)
Antopol, Tuchman and Schifrin	1937	Gasometric	Mixed	60	65 —118.5	92	(11.5)
Tod and Jones	1937	Gasometric	Mixed	30	64 —137.5	101	(18.0)
Jellinek and Looney	1939	Gasometric	Males	20 (40)	46 —179	113	31.6
McArdle	1940	Gasometric	Mixed	40	65.5—155	110	21.8
			Children	20	67.6—158	113	22.9
Faber	1941	Gasometric	Males	26			
			Females	22	55 —177	116	(30.9)
Lackey and Slaughter	1942	Titration	Mixed	50	62 —139.5	101	(17.2)
Piccoli and Longo	1947	Titration	Females	30	76 —124.5	100	12.5
Schmidt	1947	Gasometric	Mixed	12	73.5—135	104	(18.9)
Michel	1948	Gasometric	Males	42	—	—	21.5
Sawitsky, Fitch and Meyer	1948	Titration	Mixed	15	47 —157	102	30.2
Levine and Hoyt	1949	Gasometric	Males	66	—	—	20.4
			Females	67	—	—	18.9
			Females	80	—	—	22.5
Maldonado-Allende and Camponovo	1949	Titration	Males	21	77.5—138	108	13.4
			Females	29	66.5—142	104	18.6
Michel	1949	Electrometric	Males	12 (42)	—	—	21.4
Davies and Rutland	1950	Gasometric[c]	Males	49	—	—	25.8
		Electrometric	Males	47	—	—	24.5
Vorhaus, Scudamore and Kark	1950	Electrometric	Mixed	68	72.5—146	109	17.0
Scudamore, Vorhaus and Kark	1951	Electrometric	Mixed	14	65 —139.5	102	20.2

(Table 3 continued.)

Authors	Year	Technique	Sex	No. of observations	Range Mean = 100	Mid-range	Coeff. of variation
Callaway, Davies and Rutland	1951	Gasometric	Service	87	57 —143	100	19.6
			Males	76			27.1
			Females	65			23.3
Sealey	1951	Electrometric	Mixed	67	56.5—158	107	21.7
Hamblin and Marchand	1951	Electrometric	Males	11	—	—	25.8
Okinaka *et al.*	1951	Gasometric	Mixed	39	76.4—137.5	107	(14.2)
Wilson, Calvert and Geoghegan	1952	Gasometric[c]	Mixed	100	45.7—182	114	(27.2)
Wolfsie and Winter	1952	Electrometric	Males	255 (549)	45 —181	113	12.3
Aldridge and Davies	1952	Electrometric[a]	Males	230	72 —116	94	12.1
Fremont-Smith *et al.*	1952	Electrometric	Males	20	73 —148	110	25.3
			Females	20			15.4
Mann *et al.*	1952	Electrometric	Mixed	71	59.5—147	103	(18.4)
Snyder, Snyder and Bunch	1953	Titration	Males	100 (848)	61.6—123.5	92	(12.3)
			Females	125	67.8—136	102	(13.1)
Sleisinger *et al.*	1953	Colorimetric[d]	Mixed	47	65 —171	118	24.0
Sumerford *et al.*	1953	Electrometric	Males	34 (35)	53 —163	108	33.7
				22 (23)	49.5—131.5	91	42.1
				21	70 —153	112	46.0
				15 (28)	65 —137.5	101	44.0
Vogt	1953	Gasometric	Mixed	14	88 —128	108	10.6
Reinhold, Tourigny and Yonan	1953	Photometric-	Males[e] W	130	68 —125	92	15.7
			" N	46	71.5—130	101	17.2
		Electrometric	Females W	70	67 —150	109	23.1
			" N	28	79.5—139	109	19.3
Shibata and Takahashi	1953	Photometric-Electrometric	Males	20	92 —127	110	12.6
Molander, Friedman and LaDue	1954	Photometric-Electrometric	Males	110	63.5—141	102	23.3
Kaufman	1954	Titration	Males	49	—	—	29.0
			Females	51	—	—	24.4
Delcourt and van der Hoeden	1954	Colorimetric[d]	Mixed	50	70 —156	113	(19.1)
Bleisch and Shwachman	1954	Colorimetric[d]	Males and children	76	73 —128.5	101	14.6
Present paper	1955	Gasometric[f]	Males	141 (300)	72.6—138.5	106	14.7; 19.0
			Females	60 (81)	67.2—134.5	102	22.9; 26.5

a) Whole blood — b) Nine women were pregnant — c) Benzoylcholine used as substrate — d) Carbonaphthoxycholine used as substrate — e) W = white; N = negro — f) Whole blood dried on paper and butyrylcholine used as substrate — g) First value refers to the activity of plasma, second value to that of whole blood.

Table 4.

Comparison of the limits of erythrocyte acetylcholinesterase activity of healthy humans determined by various authors.

See Table 3 for comments.

Authors	Year	Technique	Sex	No. of observations	Range Mean = 100	Mid-range	Coeff. of variation
Sawitsky, Fitch and Meyer	1948	Titration	Mixed	15	83 —118	101	10.2
Davies and Rutland	1950	Gasometric	Males	50	—	—	15.4
		Electrometric	Males	48	—	—	15.9
Scudamore, Vorhaus and Kark	1951	Electrometric	Mixed	14	83.5—137.5	111	14.9
Callaway, Davies and Rutland	1951	Gasometric	Service	96	78 —122	100	10.8
			Males	79	69 —131	100	15.0
			Females	65			
Sealey	1951	Electrometric	Mixed	67	80.5—127	104	11.9
							11.6
Hamblin and Marchand	1951	Electrometric	Males	11	—	—	6.5
Wolfsie and Winter	1952	Electrometric	Males	255 (549)	51.5—160	106	10.6
Sumerford *et al.*	1953	Electrometric	Males	34 (35)	69 —145	107	40.0
				22 (23)	75.5—123	99	25.6
				21	58 —120	89	39.5
				15 (28)	85 —110	98	27.8
Kaufman	1954	Titration	Males	49	—	—	25.0
			Females	51	—	—	25.9
Present paper	1955	Gasometric[b]	Males	116 (294)	72.5—136	104	14.3; 13.3[a]
			Females	47 (97)	69.5—133	101	13.7; 15.6[a]

a) First value refers to the activity of erythrocytes, second value to that of whole blood (cf. Table 2).

b) Whole blood dried on paper and acetyl-β-methylcholine used as substrate.

and compared in Tables 3 and 4; the results reported in these tables are examples only taken from the literature rather than a complete coverage of all reports available.

There is always great difficulty when the results of experiments carried out by different techniques are compared. The only possible method of comparing the results is to convert the dispersion values of each series of experiments to a percentage form. Tables 3 and 4 are based on such conversions. The mean value of the cholinesterase activity is taken as 100 and the standard deviation as a percentage standard deviation (the "coefficient of variation"). The range is then likewise expressed. Unfortunately, many investigators have reported no values for standard devia-

tion and the only values useful for comparison are the range values recalculated as mentioned above (the mean = 100). The standard deviation can be estimated approximately from the range using Tippett's numbers (see, *e. g.*, SNEDECOR 1950). From these estimated values of standard deviation the coefficient of variation has been calculated (values in brackets in Table 3). It will be remembered that this estimation of standard deviation from the range gives the more uncertain results the larger the total number of observations.

There is a fairly good agreement in the percentage of activity variation for both types of blood cholinesterases obtained by different workers and techniques; this is especially true when the WARBURG gasometric technique and the MICHEL electrometric technique have been used. Generally, the percentage variation of the plasma (butyryl)cholinesterase activity is more pronounced than that of the erythrocyte acetylcholinesterase activity.

The higher serum cholinesterase activity in males as compared with that of females, clearly demonstrated in the present paper, has not always been established in previous investigations (see review by AUGUSTINSSON 1948; also LEVINE and HOYT 1949; CALLAWAY *et al.* 1951). To the author's knowledge, BUTT *et al.* (1942) were the first to have observed this sex difference, later confirmed by MALDONADO-ALLENDE and CAMPONOVO (1949), FREMONT-SMITH *et al.* (1952), SNYDER *et al.* (1953), REINHOLD *et al.* (1953), and by KAUFMAN (1954). As was pointed out by REINHOLD *et al.* (1953) this difference between the serum cholinesterase activity of white males and white females is expected from the known higher serum protein concentration in males than in females.

The possible dependence of serum cholinesterase activity on age has not been considered in the present investigation. This problem as well as the variation of this activity during the first months after birth have been discussed earlier by other investigators. Other factors which may be of importance in studies like the present one is the nutritional status which has an important bearing on the level of blood cholinesterase. These various factors and their influence on the variation of blood cholinesterase activity will be discussed elsewhere.

Summary.

A total of 381 determinations of plasma (butyryl)cholinesterase activity and 391 determinations of erythrocyte acetylcholinesterase activity have been carried out on whole blood samples, applied and dried on filter paper, taken from 201 healthy human individuals, 141 males and 60 females. Specific substrates were used for the two types of cholinesterases. The results are recorded in histograms of the dispersion and statistically evaluated.

The plasma cholinesterase activity of males is significantly higher than that of females. There is no such difference in the erythrocyte activity.

The results are compared with those reported by other authors.

The variation of the blood cholinesterase activity in one adult male during a period of two years is reported as an example of individual enzyme activity variation.

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Comparison of Intestinal and Skeletal Effects of Vitamin D in Relation to Dosage.

By

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It has been known for several years that vitamin D, when given in excessive doses, may cause an increased mobilization of bone salt. This action has generally been assumed to be different from the "antirachitic" effect of the vitamin, which has been believed to be due mainly to increased absorption of minerals from the gut (cf. McLEAN 1941, ALBRIGHT and REIFENSTEIN 1948, NICOLAYSEN and EEG-LARSEN 1953).

MELLANBY (1949) made the important observation in experiments on dogs, that while 20 I. U. of vitamin D daily were enough to produce a full action on the intestinal absorption of minerals, much higher doses had to be given in order to obtain a normal bone structure. The dominant rôle of the intestinal action of the vitamin therefore seemed questionable.

In experiments reported previously we observed that vitamin D even in "physiological" doses caused an increased mobilization of bone salt; this effect proved to be of primary importance for the "antirachitic" action of the vitamin, *i. e.*, the increased rate of bone-salt accretion (CARLSSON 1952, 1954, LINDQUIST 1952). These results have been recently reviewed in the light of certain observations on the mechanism of isotope fixation in bone (BAUER, CARLSSON and LINDQUIST 1955).

In view of the above-mentioned experiments of MELLANBY it was thought interesting to compare the effects of varying doses of vitamin D on the intestinal absorption of Ca and on the mobilization of bone salt.

Experimental Conditions.

Twenty-five-day-old rats were fed a modified STEENBOCK-BLACK diet with a very low Ca content (0.04 %). After 3 weeks on this diet varying doses of calciferol, dissolved in 0.5 ml peanut oil, were given by stomach tube, a control group receiving oil only. Twenty mg Ca as the lactate containing a few microC of Ca^{45} were given by stomach tube 4 days later, and the animals were killed after another 18 hours. The absorption of Ca^{45} and the serum Ca were determined. For technical details, consult earlier papers (CARLSSON 1951, 1952).

Results and Discussion.

In agreement with the experiments of LINDQUIST (1952), a dose of 10 I. U. of vitamin D was enough to give a full effect on the intestinal absorption of Ca, no further increase being observed after 100 or 1,000 I. U. (Figure 1). On the other hand, the increase in the serum Ca caused by 10 I. U. was less than half that observed after 1,000 I. U. The difference is statistically highly significant (2.3 ± 0.50 mg per 100 ml; $t = 4.6$; $DF = 13$; $P < 0.001$).

Under the present experimental conditions the serum Ca indicates the influence of vitamin D on the bone resorption (cf. CARLSSON 1952, 1954). Thus, our observations on rats are in complete agreement with MELLANBY's experiments on dogs: the response of the skeleton to vitamin D differs from that of the gut with respect to the influence of dosage.

The earlier distinction between "physiological" or "antirachitic" and "pharmacological" or "calcemic" effects of vitamin D as being caused by different mechanisms (MCLEAN 1941, NICOLAYSEN and EEG-LARSEN 1953), does not seem justified; they rather represent different degrees of action. When "physiological" doses are given, the resultant release of minerals from the resorption surfaces of the skeleton, together with the (sometimes very small) addition caused by the intestinal action, is just enough to meet the requirements of the accretion process; in this way the "antirachitic" action is brought about. If "pharmacological" doses are given, the supply of minerals from the two sources (and now the skeletal action is even more dominating, as indicated by the present experiment) will exceed the capacity of the accretion process, causing an overloading of blood and soft tissues with bone minerals, or in other words, a "calcemic" action.

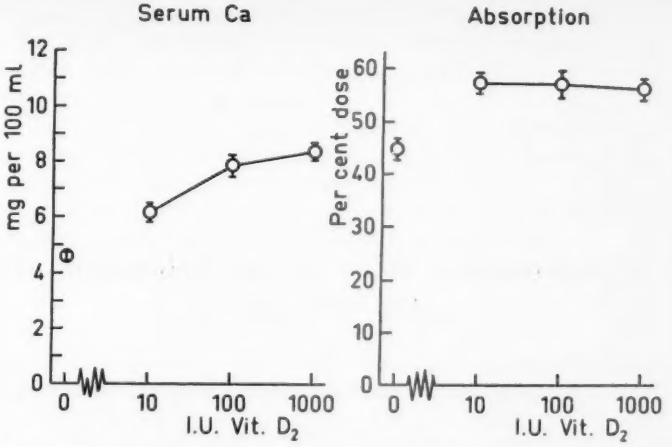

Fig. 1. The Serum Calcium and Absorption of Ca^{45} after Varying Doses of Vitamin D_2 to Rats on Low-Calcium Diet.

Symbols indicate mean ± standard error of the mean.
The number of rats in the control, 10 I. U., 100 I. U., and 1,000 I. U. groups was 10, 9, 4, and 6, respectively.

Summary.

In young rats the intestinal absorption of Ca was increased as much by 10 I. U. of vitamin D as by 1,000 I. U. On the other hand, the effect on bone resorption was much more marked after the higher dose.

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A Comparative Study on the Metabolism of Sr^{90} and Ca^{45}.

By

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The radioactive isotope Sr^{90} is generally considered to be the most dangerous of the fission products (NOONAN 1954). It has a fairly long half life, *i. e.*, 20 years. It is absorbed to a significant degree from the digestive tract. From the blood stream it is rapidly concentrated to certain distinct areas of the skeleton, which are thus exposed to radiation. The isotope is but slowly lost from the skeleton. In animals given radioactive strontium osteogenic sarcomas have been observed (KRUMHOLZ and RUST 1954).

The behaviour of strontium in the body is similar to that of calcium. However, it differs from this element in being retained to a lesser extent in the skeleton, while the excretion in both urine and feces is more rapid (NORRIS and KISIELESKI 1948). It is not known whether the higher excretion is due to the lower skeletal retention, or *vice versa*. The data of NORRIS et al. rather suggest that the former alternative is true.

The mechanism of the uptake and removal of strontium in the skeleton is obscure. It is generally agreed that physical exchange reactions in addition to accretion and resorption may be involved, though opinions differ as to the relative importance of these processes (NORRIS et al., l. c., with subsequent discussion, JONES and COPP 1951, TUTT, KIDMAN, RAYNER and VAUGHAN 1952, KID-

[1] Technical assistance: T. MAGNUSSON, M. LINDQVIST and B. JÖNSSON.

MAN, RAYNER, TUTT and VAUGHAN 1952, DUCKWORTH and HILL 1953, NEUMAN and NEUMAN 1953, RAY, VIOLETTE, BUCKLEY and MOSIMAN 1955). No data permitting a quantitative analysis of the problem seem to be available.

In a recent paper we reported a procedure for differentiating quantitatively between skeletal exchange reactions, accretion, and resorption by means of radioactive isotopes (BAUER, CARLSSON and LINDQUIST 1955 a). In the present study this procedure is employed for comparing the metabolism of Sr^{90} and Ca^{45}.

Experimental Conditions.

Two-month-old rats of both sexes, which had been fed the stock diet, were used. The diet consisted of (values in per cent):

Yellow corn	58.75
Dried skim milk	18.75
Soya been oil	6.25
Linseed cake meal	12.5
Alfalfa meal	1.5
Wheat germs	1.25
Table salt (iodized)	0.5
$CaCO_3$	0.5

At about 9 a. m. the animals were injected intraperitoneally with 1 ml of an isotonic saline solution containing 15 microC of Ca^{45} and 0.25 microC of Sr^{90}, both isotopes of a high specific activity. This proportion of activities administered was found to be the most suitable one for differentiating between the two isotopes by means of absorption, the radiation emanating from Sr^{90} (together with its daughter isotope Y^{90}) being much stronger than that emitted by Ca^{45}. The Ca^{45} was obtained from A. E. R. E., Harwell, England, and the Sr^{90}, stated to contain not more than 5 per cent of Sr^{89}, was obtained from the Radiochemical Centre, Amersham, England. At the time of injection, the urinary bladder was emptied. The animals were divided into 10 groups of 4 animals. Care was taken to make the groups as equal as possible with respect to litter, sex and body weight. The different groups of animals were killed at varying intervals ranging from 15 minutes to 48 hours after injection. Immediately after injection each of the animals intended to survive for 4 hours or more was placed in an individual cage provided with an arrangement for separation of urine and feces. Before these animals were killed, their bladders were emptied. After death the bladder was examined in each case. In two rats there still remained some urine which was added to the urine previously excreted. In the animals allowed to survive less than 4 hours urine and feces were not collected. Food was available from 8 hours after injection. However, none of the animals of the 12-hour group took

any food, in accordance with the fact that the food intake of rats is largely confined to the night. Later on, the animals allowed to survive more than twelve hours appeared to consume normal amounts of food.

The animals were killed by bleeding under ether anesthesia. Serum Ca was determined according to CLARK and COLLIP (1925). After titration, the supernatant serum, the washings, and the titrated calcium oxalate solution from each animal were pooled, and 1.5 ml of a 5 per cent solution of $Sr(NO_3)_2$ was added. Excess ammonium oxalate was then added, and the samples were left overnight. The specimens were then centrifuged. The supernatants were still found to be radioactive, owing to the fairly high solubility of strontium oxalate. One ml of the $Sr(NO_3)_2$ solution was added and the precipitation was repeated. The supernatants were then free of activity. The two precipitates were pooled and dissolved in 2.5 ml HNO_3, density 1.10.

The gastrointestinal tract (together with the fecal collection), the ends and shafts of the pooled right and left tibia-fibulas (in the following called tibias) of each animal, and the four incisors (after removing the pulp) were ashed in an electrically heated muffle, while the urine was ashed by means of H_2SO_4 and HNO_3. An aliquote of the urine was preserved for Ca analysis, which was done according to the same principles as the determination of the serum Ca.

In order to re-establish equilibrium between Sr^{90} and its daughter isotope Y^{90} (half life 65 hours), 20 days were allowed to pass after the sacrifice of the animals before the samples were counted. Counting was made on liquid samples (2 ml) after suitable dilution with HNO_3, density 1.10. Each sample was counted with and without an aluminium absorber, which absorbed all the Ca^{45} radiation and 41 per cent of the Sr^{90}, Y^{90} radiation. The activity emanating from Ca^{45} and Sr^{90}, Y^{90}, respectively, was calculated and compared with the activity of standard solutions of either of the two isotopes; the values obtained were expressed as per cent of the dose administered.

For further details on the collection, pretreatment, and counting of the samples, see CARLSSON (1951).

All the samples of one rat belonging to the 1/2-hour group contained only about one third of the activity of the remaining animals of the group. Possibly the injection had been made partly or entirely into the bladder or the gastrointestinal tract (urine and feces were not collected at this time interval). The activity data of this animal have been omitted, even though they would probably have been without any detectable influence on the results of the entire material. — One of the feces samples of the 24 hour group, one of the incisors samples and one of the urine samples of the 48-hour group were lost.

Principles of Computation.

The amount of an isotope such as Ca^{45} present in a calcified tissue can be written (cf. BAUER, CARLSSON and LINDQUIST 1955 a)

$$Ca^{45}_{Obs} = Ca^{45}_{E} + Ca^{45}_{A} - Ca^{45}_{R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Ca^{45}_{Obs} = total amount of Ca^{45} present in a calcified tissue.

Ca^{45}_{E} = amount of Ca^{45} present in the exchangeable fraction of the bone salt.

Ca^{45}_{A} = amount of Ca^{45} incorporated into the nonexchangeable fraction of the bone salt through accretion.

Ca^{45}_{R} = amount of Ca^{45} removed through resorption.

Under the assumption that the specific activity $\left(= \frac{Ca^{45}}{Ca^{45} + Ca^{40}}\right)$ of the exchangeable Ca fraction rapidly attains the same value as the specific activity of the plasma Ca and that these two specific activities can then be regarded as equal (cf. BAUER, CARLSSON and LINDQUIST 1955 b), we get

$$Ca^{45}_{E} = E \times S \quad \ldots\ldots (2)$$

E = amount of Ca ($= Ca^{40} + Ca^{45}$) in the exchangeable fraction of the bone salt.

S = specific activity of the serum (plasma) Ca.

Since it must be assumed that Ca^{45} and Ca^{40} are deposited in the bone salt in the same proportions as they simultaneously occur in the blood, we get

$$Ca^{45}_{A} = A \times T \times S_{M} \quad \ldots\ldots (3)$$

A = rate of Ca accretion.

T = interval of time between administration of Ca^{45} and observation.

S_M = *average* specific activity of serum Ca during this time interval.

Combination of equations (1), (2), and (3) gives

$$Ca^{45}_{Obs} = E \times S + A \times T \times S_{M} - Ca^{45}_{R} \quad \ldots\ldots (4)$$

At sufficiently short intervals Ca^{45}_{R} can be disregarded. From observations made at two different intervals T_1 and T_2 we then get

$$Ca^{45}_{1\text{-}Obs} = E \times S_1 + A \times T_1 \times S_{1\text{-}M} \quad \ldots\ldots (4:1)$$

$$Ca^{45}_{2\text{-}Obs} = E \times S_2 + A \times T_2 \times S_{2\text{-}M} \quad \ldots\ldots (4:2)$$

From these two equations we obtain

$$\frac{Ca^{45}_{1\text{-}Obs} - A \times T_1 \times S_{1\text{-}M}}{Ca^{45}_{2\text{-}Obs} - A \times T_2 \times S_{2\text{-}M}} = \frac{S_1}{S_2} \quad \ldots\ldots (5)$$

After A has been calculated, E can be obtained by introducing the calculated A value in equation (4:1).

Equation (5) can be used for computing the accretion rate not only in different calcified tissues but also in the whole skeleton. In this computation the whole body is treated in the same manner as a calcified tissue. Ca^{45}_{Obs} is then equal to 100 minus per cent of dose lost *via* the kidneys and the gastrointestinal tract, and E is the amount of Ca present in the exchangeable fraction of the bone salt *plus* the amount of Ca of the fluid phase of the body.

The Sr^{90} data were treated in exactly the same way as the Ca^{45} data. For both isotopes the blood activities were thus expressed as per cent of dose per mg Ca. Evidently this procedure permits the various meta-

bolic processes to be studied with respect to similarities or differences between the two elements. It should perhaps be noted that the activity per mg Ca was, on an average, equal to the activity per 10 ml serum, the average concentration of Ca in the entire material being 10.0 mg per 100 ml. (The standard error of the single observation was 0.39 mg per 100 ml.)

Results.

Blood Activities. The Ca^{45} and Sr^{90} activity curves of the blood serum had the same general shape (Figures 1 and 2). The Sr^{90} activity was somewhat lower than the Ca^{45} activity, with the possible exception for longer intervals. (The curves seemed to intersect about 24 hours after injection. However, the number of observations does not seem large enough to establish this finding.) Consequently, $T \times S_M$ was about 15 per cent lower for Sr^{90}.

Excretion. About 13 per cent of the injected Sr^{90} was recovered from the urine during the first 24 hours after injection, as compared with 0.9 per cent of the injected Ca^{45} (Figures 3 and 4). A similar, though less marked difference in fecal excretion was observed, the 24-hour values being 10 and 5 per cent, respectively. This difference in the activity of the gastrointestinal tract plus the feces seemed to be less pronounced at shorter intervals, suggesting that intestinal reabsorption rather than excretion (or secretion) was involved; however, this problem seems to require further study.

As the activity per mg Ca in the serum was lower for Sr^{90} than for Ca^{45} it is evident that the clearance of Sr^{90} was higher than that of Ca^{45} both in the kidneys and to a lesser degree, in the digestive apparatus, both of which are thus able to distinguish between the two elements.

Retention. As a consequence of the higher excretion figures of Sr^{90} the retention of this isotope in the whole body (Figures 3 and 4) and in the calcified tissues (Figures 5 and 6) was lower than that of Ca^{45}.

Accretion Rate. The same value for the accretion rate of Ca in the whole skeleton was obtained whether calculated from the Sr^{90} or Ca^{45} data according to equation (5) (Table I). This was essentially true also for the values of accretion rates in the various calcified tissues.

Exchange Reactions. Practically the same values for the size of the exchangeable fraction of Ca were obtained from the Sr^{90} and

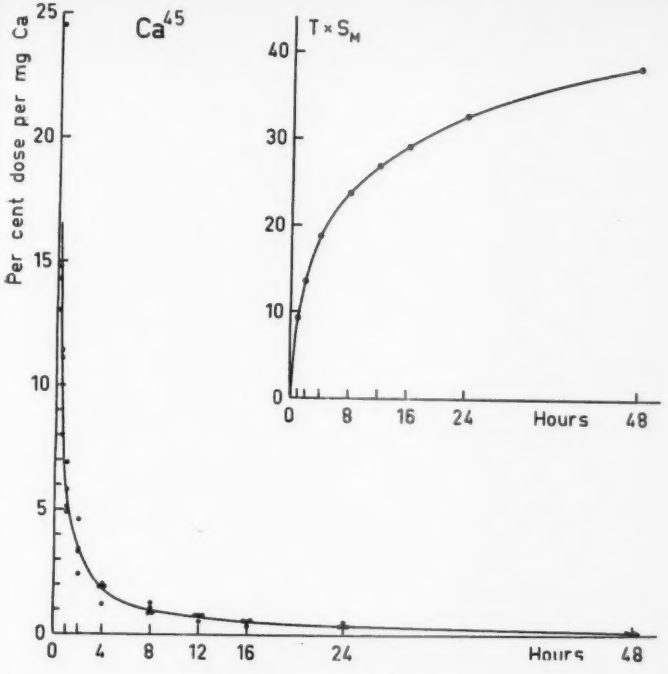

Fig. 1. Ca^{45} Activity of Blood Serum.
The values are expressed in per cent of dose per mg Ca. $T \times S_M$ = Area covered y the serum activity curve.

Ca^{45} data in the body as a whole as well as in the calcified tissues, with the possible exception for the tibia shafts, in which a higher value was derived from the Sr^{90} data (Table I). Whether this is a real difference or is due to experimental error cannot be judged from the available data. In the incisors the exchangeable fraction was very small and could not be estimated with any great accuracy.

The Influence of Resorption. The agreement between calculated ($Ca^{45}_E + Ca^{45}_A$) and observed (Ca^{45}_{Obs}) values was good, except for intervals longer than 16 hours, when the values observed in the whole skeleton and in the ends of the tibias were lower than the calculated values. This is reasonably due to resorption (BAUER et al. 1955 a, l. c.). Whether the two isotopes differ significantly with respect to resorption cannot be judged from the present

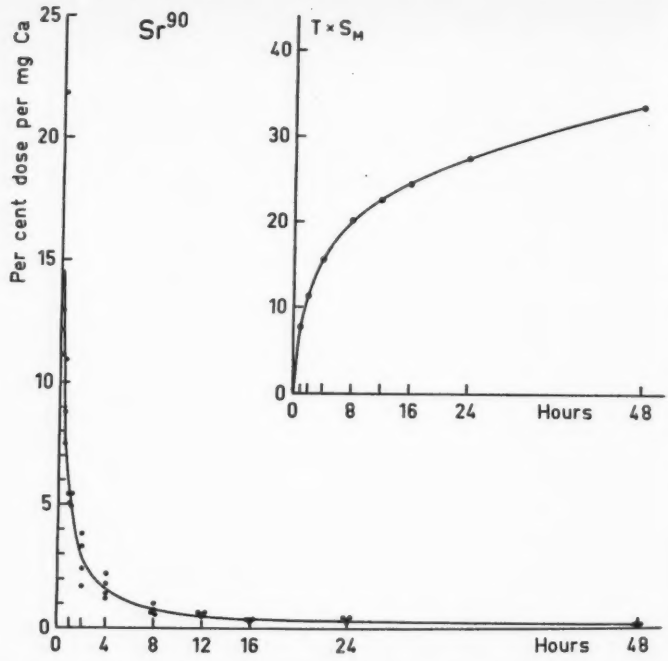

Fig. 2. Sr^{90} Activity of Blood Serum.

The values are expressed in per cent of dose per mg Ca. $T \times S_M$ = Area covered by the serum activity curve.

Table I.

Skeletal Metabolism of Sr^{90} and Ca^{45} in Young Rats.

	Accretion rate, mg Ca per hour, from		Exchangeable Ca, mg, from		Ca content, mg
	Ca^{45}	Sr^{90}	Ca^{45}	Sr^{90}	
Whole skeleton	2.7	2.7	28[1]	31[1]	1,000[2]
Tibias, ends	0.18	0.16	1.6	1.9	35
Tibias, shafts	0.038	0.032	0.3	0.6	27
Incisors	0.080	0.074	0.1	0.2	44

[1] This figure includes the Ca of the soft tissues of the body.
[2] Roughly estimated from body weight (120 grams).

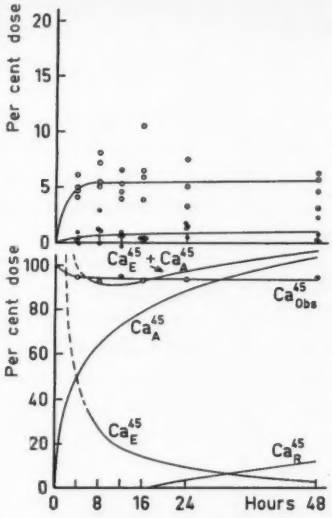

Fig. 3. Excretion and Over-All Skeletal Metabolism of Ca^{45}.

Upper graph: circles indicate Ca^{45} in gastrointestinal tract *plus* feces; dots indicate Ca^{45} in urine.

Lower graph: circles indicate retention of Ca^{45} in the whole body (mean values). For explanation of symbols, see under "Principles of Computation".

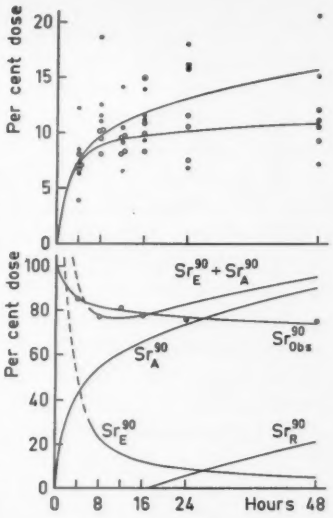

Fig. 4. Excretion and Over-All Skeletal Metabolism of Sr^{90}.

Upper graph: circles indicate Sr^{90} in gastrointestinal tract *plus* feces; dots indicate Sr^{90} in urine.

Lower graph: circles indicate retention of Sr^{90} in the whole body (mean values). For explanation of symbols, see under "Principles of Computation".

data; in view of the other findings a difference would hardly be anticipated.

Thus, neither with regard to accretion nor to exchange the skeleton appears capable of distinguishing between Sr and Ca. So far, however, this conclusion is valid only for conditions where Sr is present in tracer amounts. Further investigation is required to settle whether this is true also when larger quantities of Sr are present.

Discussion.

The lower retention of Sr^{90} compared with Ca^{45} was found to be essentially due to preferential excretion (or failure of reabsorption) of the former element in the kidneys and in the gastrointes-

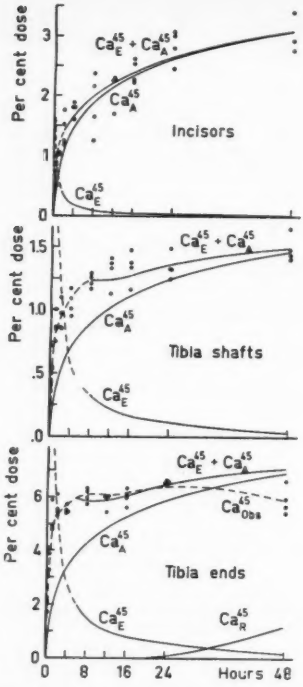

Fig. 5. Rôle of Accretion and Exchange Reactions in the Uptake of Ca^{45} in Various Calcified Tissues.

Dots indicate single observations (Ca_{Obs}^{45}). For explanation of symbols, see under "Principles of Computation".

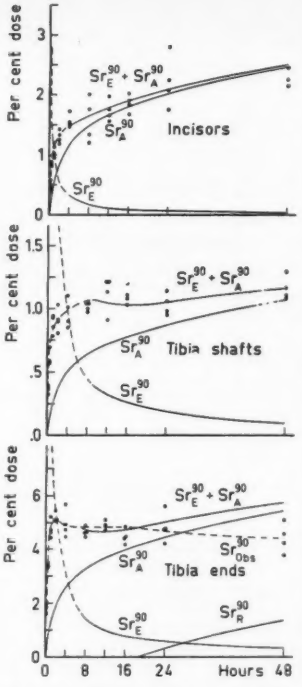

Fig. 6. Rôle of Accretion and Exchange Reactions in the Uptake of Sr^{90} in Various Calcified Tissues.

Dots indicate single observations (Sr_{Obs}^{90}). For explanation of symbols, see under "Principles of Computation".

tinal tract, the skeleton apparently being unable to distinguish between these two alkaline earths.

The importance of early treatment of Sr^{90} poisoning is emphasized by its rapid incorporation into the nonexchangeable fraction of the bone salt. In the present experiment about half of the isotope present in the skeleton 4 hours after injection had been fixed in this way. Naturally, species differences may exist, so that information on the exact timing of events in humans cannot be derived from animal experiments. It does not seem unlikely that this problem can be studied in humans. It is true that Ca^{45} and

Sr^{90} have too long half lives for human experiments, and P^{32} cannot be used for calculations of the accretion rate in the whole skeleton, since all the extraskeletal P^{32} is not freely exchangeable. However, the virtual absence of a difference in the skeletal metabolism of Sr^{90} and Ca^{45} arouses the suspicion that other isotopes with more suitable half lives may prove employable. Thus the data of HAMILTON (1947) suggest that the metabolic behaviour of Ba and Sr is very similar. Carrier-free Ba^{140}, with a half life of 12.8 days, is available. The data at hand suggest that this isotope may become a useful tool in the study of skeletal function in man.

If no marked difference exists between rats and humans as regards the relative importance of accretion and exchange (and our P^{32} data do not suggest the existence of such a difference, BAUER, CARLSSON and LINDQUIST 1955 c), it may be concluded that therapeutic measures in Sr^{90} poisoning should be directed towards increasing excretion (cf. JONES and COPP l. c.) and lowering skeletal accretion.

Summary.

The distribution and excretion of Sr^{90} and Ca^{45} was studied at varying intervals after simultaneous intraperitoneal injection of the isotopes to young rats.

Sr^{90} was retained to a lesser extent than Ca^{45} owing to the preferential excretion (or failure of reabsorption) of the former element by the kidneys as well as by the gastrointestinal tract.

With regard to skeletal accretion and exchange there was no marked difference between the two elements. The importance of the accretion process for the fixation of the isotopes in the skeleton is emphasized.

The computation of the rate of Ca accretion in the whole skeleton is described.

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Some Properties of the Exchangeable Bone Calcium.

By

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Besides the true skeletal anabolism and katabolism, physical exchange reactions occur between the bone salt and the surrounding fluid phase. It has been suggested that these reactions are of two kinds, *i. e.*, "surface exchange" (or "ionic exchange") and "recrystallization"; in the former type of reaction the ions participating in the exchange are present on the surfaces of the bone salt crystals, while in the latter type they are more deeply situated (*cf.* NEUMAN and NEUMAN 1953). In addition to these processes exchange between the free or complex-linked ions of the fluid phase of the bone and those of the blood should be considered; it may be of some importance at very short intervals after injection of an isotope such as Ca^{45}.

As pointed out by BAUER (1954), earlier evaluations of the exchange reactions of the bone salt *in vivo* left out of consideration the accretion and resorption processes. In the experiments reported below this source of error is controlled. It is shown that the exchangeable bone Ca is not uniform but probably consists of several subfractions.

Experimental Conditions and Principles of Computation.

The data used for the computations are taken from a paper of BAUER, CARLSSON and LINDQUIST (1955 a), in which the distribution and excretion of simultaneously intraperitoneally injected Ca^{45} and

Sr^{90} were studied in two-month-old rats. Only the Ca^{45} data are dealt with in the present paper; there were no important differences between the two isotopes as regards skeletal metabolism. Intervals were considered, at which the influence of bone resorption could be disregarded. The exchangeable bone Ca^{45} (Ca^{45}_E) is then equal to the difference between the total Ca^{45} present in the bone (Ca^{45}_{Obs}) and the Ca^{45} incorporated into the nonexchangeable fraction of the bone salt through accretion (Ca^{45}_A), or

$$Ca^{45}_E = Ca^{45}_{Obs} - Ca^{45}_A.$$

The calculation of Ca^{45}_A was described in an earlier paper (BAUER, CARLSSON and LINDQUIST 1955 b). Ca^{45}_E was divided by E (amount of exchangeable bone Ca; for calculation, see our earlier paper); in this way the specific activity of the exchangeable bone Ca was obtained.

Results and Discussion.

The specific activity of the exchangeable fraction of bone Ca at varying intervals after injection of the isotope is given for the tibia shafts in Figure 1.

If the exchangeable fraction of bone Ca were homogeneous, the specific activity of this fraction would rise to a maximum where it would be equal to the specific activity of the serum Ca. Thereafter the specific activity of the exchangeable Ca would follow the dropping specific activity of the serum Ca, though at a slightly higher level. In Figure 1 the specific activity of the exchangeable bone Ca does not show such a behaviour. It has a maximum of 1.5 per cent of dose per mg Ca about one hour after injection, when the specific activity of the serum Ca is 5.7 per cent of dose per mg Ca. The subsequent course of the activity curve is irregular and does not reproduce the shape of the blood activity curve. It appears to have a plateau somewhere between 4 and 8 hours after injection. Intersection with the specific activity of the serum Ca occurs after about eight hours. It is evident that the exchangeable Ca in this calcified tissue is not uniform. One subfraction of the exchangeable Ca apparently exchanges rapidly with the blood Ca and attains the specific activity of the serum Ca in about one hour; in another, slowly exchanging subfraction this does not occur until about eight hours after injection. Attempts have been made to divide the total exchangeable bone Ca into a small number of subfractions. However, the fits obtained were not satisfactory. It therefore

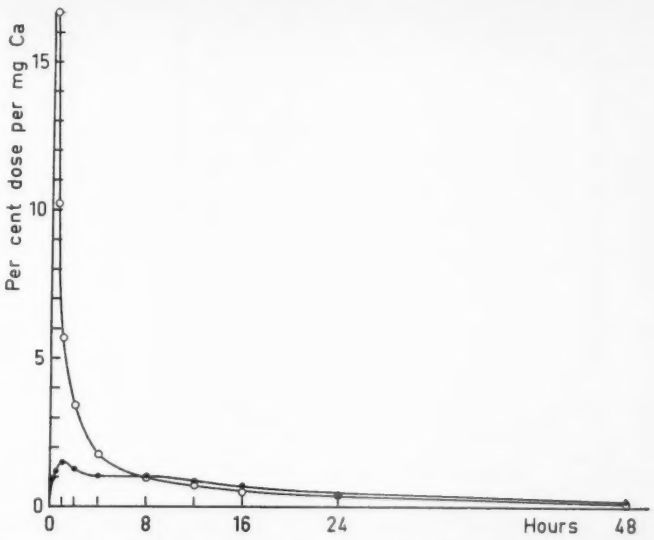

Figure 1. Specific Activities of the Exchangeable Ca of the Tibia Shafts (Dots) and of the Serum Ca (Circles) at Varying Intervals after Injection of Ca^{45}.

seems most reasonable to assume that the number of subfractions is very large, or rather that we are dealing with a continuous series of subfractions with varying rates of exchange.

Similar findings were made in the tibia ends (Figure 2). The over-all rate of exchange seemed to be somewhat more rapid than in the shafts. The specific activity of the serum Ca was attained about six hours after injection. The whole skeleton behaved similarly (Figure 3). The crossing with the specific activity of the serum Ca occurred after about six hours.

It is interesting to see how the shape of the specific activity curve of the serum Ca is influenced by exchange, accretion, and resorption. In Figure 4 the serum specific activity is plotted against time, the ordinate being logarithmical. It is seen that between 6 and 16 hours after injection a straight line is obtained. At shorter intervals the slope is steeper, at longer intervals less steep. This shape of the curve may be interpreted as follows. After the exchangeable skeletal Ca has attained the specific activity of the serum Ca, the drop in the blood Ca^{45} will be caused by two different mechanisms, *i. e.*, skeletal accretion, and ex-

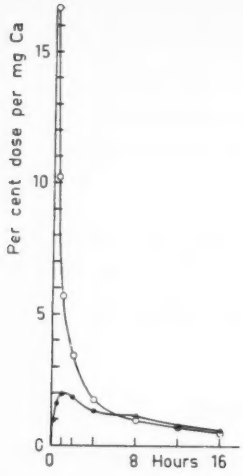

Figure 2. Specific Activities of the Exchangeable Ca of the Tibia Ends (Dots) and of the Serum Ca (Circles) at Varying Intervals after Injection of Ca^{45}.

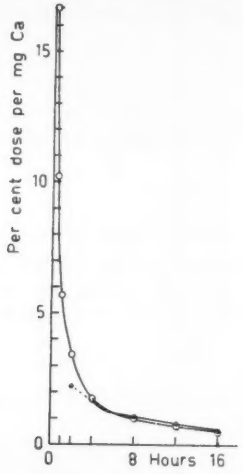

Figure. 3. Specific Activities of the Total Exchangeable Ca of the Body (Dots) and of the Serum Ca (Circles) at Varying Intervals after Injection of Ca^{45}.

cretion by different routes. Since these processes are irreversible, the serum specific activity will drop at a rate proportional to its own level, or in other words, a straight line will be obtained, if the ordinate is logarithmic. However, this will be true only as long as the Ca entering the blood stream from the resorption surfaces of the bones does not contain any Ca^{45}. When resorption of Ca^{45} sets in, there will be a new break in the blood activity curve. This break is seen to occur about 16 hours after injection. (A distorsion of the blood activity curve caused by the reabsorption of Ca^{45} from the gut would perhaps be anticipated. One reason why no such distorsion is detectable, may be that the intestinal secretion and reabsorption of Ca are small compared to skeletal accretion and resorption.)

It may be concluded that the exchangeable Ca of the skeleton consists of a (probably large) number of subfractions attaining the specific activity of the serum Ca at intervals ranging in the young rats of the present investigation from about one to about six or eight hours. With the accumulation of new experimental

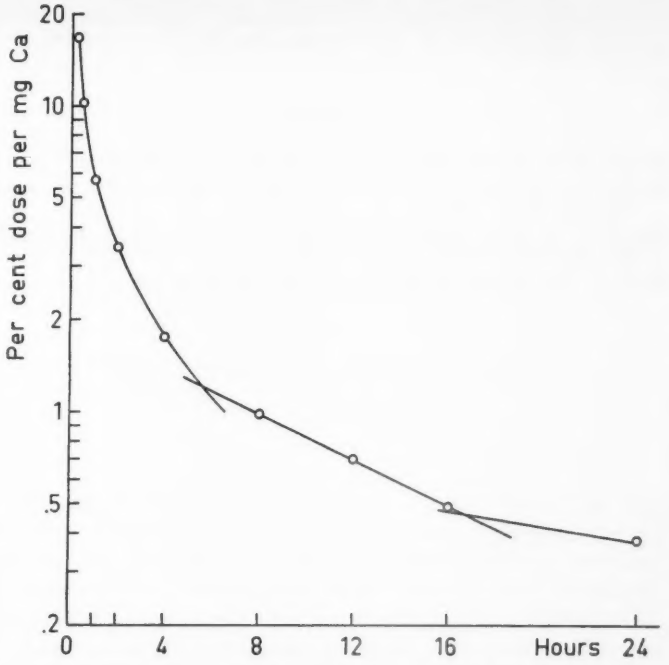

Figure 4. Specific Activity of the Serum Ca at Varying Intervals after Injection of Ca^{45}.

Note. The scale of the ordinate is logarithmic.

material we hope to obtain more accurate information, based on a strict mathematical analysis.

The data indicate that the lag in the specific activity of the exchangeable bone Ca relative to the specific activity of the serum Ca is fairly slight. It therefore does not imply any great error to set the lag to zero after the point of intersection has been reached. It should perhaps be recalled, that this procedure gives a maximum value of the amount of exchangeable bone Ca. The greater the lag, the smaller will be the value for the exchangeable Ca fraction.

Summary.

In young rats the exchangeable fraction of the bone Ca was found to consist of a (probably large) number of subfractions

attaining the specific activity of the serum Ca at intervals ranging between one and six or eight hours after injection of Ca^{45}.

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The Cardiac Output in Rest and Work at Low and High Oxygen Pressures.

By

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Previous determinations of the cardiac output on normal man at low and high oxygen pressures have been made mainly by the foreign gas methods (*e. g.* HASSELBALCH and LINDHARD 1915, GROLLMAN 1930, CHRISTENSEN et al. 1936 and 1937, ASMUSSEN et al. 1941 a and b), by the ballistocardiographic method (*e. g.* DOUGLAS et al. 1919, WHITEHORN et al. 1946, OTIS et al. 1946) and by the direct FICK method by means of heart catheterization (*e. g.* MOTLEY et al. 1947, WESTCOTT et al. 1951, STORSTEIN 1952), although also other methods have been used (pulse wave methods, roentgenkymographic methods and the CO_2 equilibration method).

The general outcome of the above mentioned studies is that the resting cardiac output is elevated at *low oxygen pressures*, but as to the magnitude of this elevation the results show great variances even at the same degree of oxygen want. One reason for the wide scattering of the results is presumably that the foreign gas methods when used at low oxygen pressures will tend to give too high values of the cardiac output. When using the foreign gas method it is a necessary condition that the oxygen content of the arterial blood does not change during the sampling time. As in low oxygen the arterial oxygen content is decreasing throughout the period of rebreathing it has often been used to check the results by comparing the arithmetric mean oxygen tension during the sampling time with the alveolar oxygen tension existing previous to the rebreathing. But since the oxygen uptake from

the lung-bag system to the blood due to the shape of the oxygen dissociation curve is relatively larger in the beginning of the sampling period than later, the arithmetric mean oxygen tension can be considerably higher than the virtually existing mean oxygen tension. As a consequence the arterio-venous oxygen difference will be determined too low when the arithmetric mean oxygen tension is used as a control. Also methods by which it has been attempted to use the oxygen uptake during the sampling period as a control are encumbered with uncertainties and technical difficulties.

While objections thus can be raised against using the foreign gas methods at low oxygen pressures it seems reasonable to assume that the direct FICK method also in this condition can give reliable results. However only few series of experiments with this method on normal resting man in low oxygen are available and none during muscular work. MOTLEY, COURNAND et al. (1947) in 5 normal subjects breathing 10 per cent oxygen (HbO_2 saturation 73 per cent) found that the arteric-venous oxygen difference decreased from an average of 42 cc per liter to 37 cc per liter, *i. e.* a decrease of 11.9 per cent. The pulse rate increased 19.4 per cent, but it was found that the cardiac output nevertheless had decreased 9.4 per cent. The reason for this was as also later admitted by COURNAND (1950) that a steady state of oxygen uptake had not been reached. Notwithstanding the found decrease in cardiac output, the decrease of 11.9 per cent in the arterio-venous oxygen difference shows that the cardiac output must have been increased in relation to the oxygen uptake.

WESTCOTT et al. (1951) determined the cardiac output and the arterio-venous oxygen difference in 11 normal subjects breathing 13 per cent oxygen (HbO_2 saturation 79 per cent) and compared them to controls determined before and after the breathing of the low oxygen mixture. The authors found no statistically significant change in the cardiac output. But when the low oxygen values are compared to the *last* of the control values where an approximate steady state of oxygen consumption was reached, the average arterio-venous oxygen difference shows a decrease of 11.4 per cent and the cardiac output an increase of 12.2 per cent. STORSTEIN (1952) had 12 normal subjects breathing air with an oxygen content of 9.5 to 15 per cent and found that on an average the arterio-venous oxygen difference was 31.7 cc per liter as compared to 35.0 when the subjects were breathing ordinary air. How-

ever, if the material is divided in two groups, one with oxygen saturations above 80 per cent (mean 86 per cent) and one with oxygen saturations below 80 per cent (mean 66 per cent) it appears, that in the former group the arterio-venous oxygen difference is not decreased but rather the opposite whereas in the second group the arterio-venous oxygen difference is decreased from 36 cc per liter in air to 26 cc per liter in low oxygen. The determinations, however, scatter considerably around the mean.

The rather scanty available data from normal subjects thus seem to indicate that the arterio-venous oxygen difference is somewhat decreased in low oxygen. This finding is corroborated by studies on patients in which it was found that a good correlation existed between the arterial oxygen unsaturation and the cardiac output, especially below a critical level of 82 per cent arterial oxygen saturation. (cp. COURNAND 1950).

Studies of the effect of *high oxygen* on the cardiac output are but few. For technical reasons the foreign gas methods are difficult and less reliable at oxygen percentages above 40—45 and the method cannot be used in pure oxygen. OTIS et al. (1946) using the ballistocardiograph found on five subjects that in pure oxygen the pulse rate was decreased by 8.5 per cent, and that the ballistocardiographic records, which tentatively were interpreted in terms of cardiac output, likewise showed a decrease of 8.5 per cent. WHITEHORN et al. (1946) concluded from ballistocardiographic studies on 12 to 27 subjects that breathing of pure oxygen had decreased the cardiac output of their subjects by 15.5 per cent. The pulse rate had decreased from 8 to 13.9 per cent.

While the absolute values of the cardiac output obtained by the ballistocardiographic method mostly have been considered doubtful it has often been assumed, that it gave true relative values. Investigations by CATHCART et al. (1953), however, have shown by comparison with the direct FICK method that not even this is the case.

Data on the cardiac output in normal resting subjects breathing pure oxygen obtained by mean of the direct FICK method have been published by STORSTEIN (1952). His 12 subjects showed an average arterio-venous oxygen difference of 35.0 cc per liter when breathing air and 36.3 cc per liter when breathing pure oxygen. The pulse rate decreased from 85 in air to 81 in oxygen.

It was the purpose of the present investigation to study the effect of changes in the oxygen pressure in the inspired air on the

cardiac output in rest and especially during muscular work in an attempt to elucidate the mechanisms by which the circulation is regulated. Further it was intended to study the effect of high oxygen on the cardiac output in maximum work in which it previously has been found (NIELSEN and HANSEN 1937) that the oxygen uptake can be increased considerably above what was possible in ordinary air.

Methods and Procedure.

The cardiac outputs were determined by the dye injection method. The reliability of this method must be assumed to be just as good at low and high oxygen pressures as under normal conditions.

The work experiments were performed at the KROGH bicycle ergometer, which had been fitted out with a special seat allowing the arms of the subjects to be free and relaxed for the cardiac output determinations. The dye (T-1824) was injected into a left cubital vein and the arterial samples were taken from the right brachial artery. The handling of the samples etc. has been described in detail in an earlier paper (ASMUSSEN and NIELSEN 1952). Arterial blood samples for determination of the arterial oxygen saturation were subsequently collected in ice cooled sampling tubes under oil and after mixing with heparin transferred to calibrated 1 cc syringes. These were kept on ice and the blood was transferred directly from the syringes to the VAN SLYKE apparatus for analyses.

The determination of the oxygen uptake was made by the DOUGLAS-bag method in all the experiments with low oxygen and in the corresponding control experiments in air. The low oxygen mixtures were inspired from a 120 liter DOUGLAS-bag, which was continuously refilled from a cylinder containing a mixture of about 12 per cent oxygen in nitrogen. The gas analyses were made on the HALDANE apparatus or on the SCHOLANDER apparatus.

In the experiments with pure oxygen and in the corresponding control experiments in air the oxygen uptake was determined by means of a closed system. One difficulty in using a closed system during heavy work in which the CO_2 elimination may exceed 3 to 4 liters of CO_2 per minute is to secure a complete CO_2 absorption without producing increased resistance against the respiration. This difficulty was overcome by letting the expired air pass through a pre-absorber consisting of 32 rotating discs of stainless steel dipping down into a strong solution of sodium hydroxide. Having passed between these wetted discs the expired air was led through three inlets into a wide (about 80 cm diameter) and low (about 20 cm) soda lime container. The oxygen taken up by the subject was replenished automatically from two cylinders through two registering wet gas meters. One of these was kept running continuously and delivered the larger part of the oxygen while the other was connected to the cylinder through a thin-walled rubber

tube the opening and closing of which was effected by means of an electro magneto, governed by a registering KROGH spirometer so that the volume of the closed system could be kept constant. The oxygen uptake could then be determined from the recordings of the two gas meters.

The experiments were performed on healthy young male students of average physical fitness. The subjects were fasting in the rest experiments whereas in the work experiments a light breakfast was allowed, but all fat was prohibited so as not to interfere with the dye determination in the plasma. All determinations were performed in the steady state period after 15 to 20 minutes of work.

Results.

a. Low oxygen pressure.

The results from the experiments in low oxygen are presented in fig. 1. It can be seen that the cardiac output increases with increasing oxygen uptakes and that the values from the experiments in low oxygen are higher than those from the air experiments. The arterio-venous oxygen differences also increase with increasing oxygen uptake and it can be seen that the values are lower when the oxygen content of the inspired air is reduced. The comparatively large scattering of the values and the fact that the values are not distributed around smooth curves are caused by the various state of physical training of the subjects. For instance the relatively large increase in cardiac output at the highest work intensities, and the corresponding low arterio-venous oxygen differences, are explained by the fact, that only the best trained subject was able to work in low oxygen at this high intensity of work. A review of the available data from the literature have shown that well trained subjects in heavy work are able to reach and maintain a larger cardiac output than less well trained subjects at the same intensity of work (ASMUSSEN and NIELSEN 1955).

The relative changes in the circulatory functions in rest and work that were produced by breathing air mixtures with a low oxygen pressure are presented in table 1.

The table shows that the cardiac output is from 10 to 20 per cent higher and the arterio-venous oxygen difference from 10 to 20 per cent lower in the experiments with low oxygen than in the control experiments. The pulse rate was increased somewhat more than the cardiac output and the stroke volume was consequently somewhat lower in the low oxygen experiments.

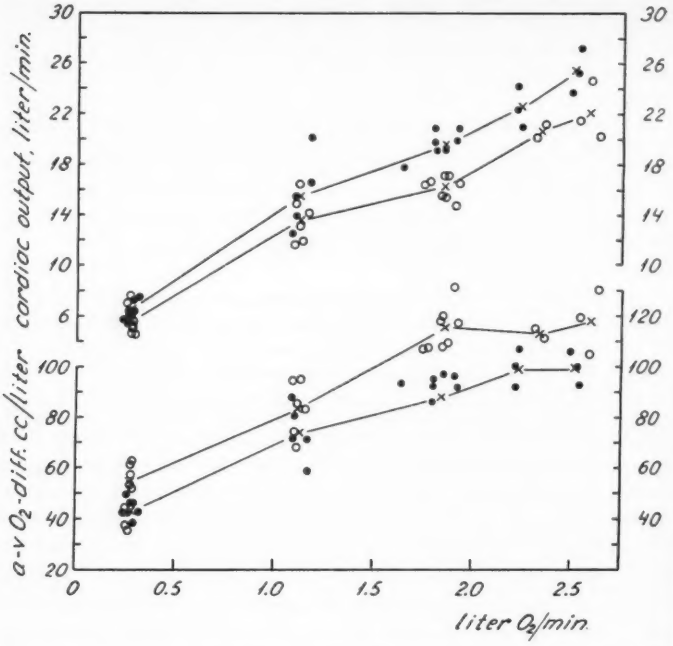

Fig. 1. Cardiac output and arterio-venous oxygen difference in rest and work in relation to oxygen consumption.

○ experiments in air
● experiments in low oxygen
The lines connect the average values (×) for the different work intensities.

b. Pure oxygen.

The results from the experiments with pure oxygen and the corresponding control experiments in air are presented in table 2. It appears from the table that the oxygen uptake has been the same in the two groups of experiments at all work intensities except at the very highest work intensity at which only one control experiment was performed. The oxygen consumption in the control experiments at the work intensity of 900 mkg per minute was determined by the DOUGLAS-bag method. From the close agreement between the O_2 uptakes in the air and in the O_2 experiments found by means of the closed system method at the other work intensities it is reasonable to assume that the oxygen uptake also at 900 mkg per minute has been the same in pure oxygen as

Table 1.

Average percentage changes in circulatory functions in low O_2 (about 12 per cent O_2 in nitrogen, HbO_2-saturation 65—70 per cent).

Intensity of work mkg/min	Cardiac output	Pulse rate	Stroke volume	Arterio-venous O_2-diff.
0	+ 16 %	+ 31 %	— 11 %	— 15 %
360	+ 13 %	+ 16 %	— 2 %	— 11 %
720	+ 21 %	+ 16 %	+ 4 %	— 19 %
900	+ 12.5 %	+ 18 %	— 5 %	— 15 %
1,080	+ 10 %	+ 22 %	— 10 %	— 14 %

in air. The cardiac outputs and the arterio-venous oxygen differences were found to be nearly the same in the air experiments as in the pure oxygen experiments. At the work intensities for which a sufficient number of experiments is available the standard error of the means are presented in the table. These standard errors include both the individual variations and the scattering which is due to the method. It can be seen from the mean values and their errors that the small differences existing between the two series of experiments are not statistically significant. Even when the air and the oxygen values from the single individuals are compared the differences are so small and inconsistent that an effect of oxygen breathing on the size of the cardiac output can not be seen. The pulse rate, on the other hand, shows a small but systematic decrease in the oxygen experiments. The average decrease in pulse rate is found to be about 6 per cent.

Discussion.

The experiments in low oxygen have shown, that the cardiac output is increased 10 to 20 per cent when the arterial oxygen saturation is diminished to 65 to 70 per cent. This result is in agreement with the results from earlier resting experiments in low oxygen, in which the direct FICK method was employed. The increase is, however, lower than that found with the foreign gas method both in rest and during exercise. The reason for this discrepancy has been discussed earlier (see page 73).

The increase in cardiac output is produced by an increase in the pulse rate whereas the stroke volume rather undergoes a slight decrease. The pulse acceleration both in the rest and in the work

Table

Work intensity mkg/min	Control experiments in air					
	Number of experiments	O_2-uptake l/min	Cardiac output l/min	a-v O_2-diff. cc/l	Pulse rate	Stroke volume cc
900	8	2.18	18.9 ± 0.97	117	151	125
1,080	16	2.50	19.4 ± 0.65	132	165	118
1,260	10	2.89	21.2 ± 0.73	137	167	127
1,440	4	3.28	22.4	147	177	127
1,620	1	3.76	27.7	136	166	167

experiments is of the same magnitude as that found in corresponding experiments in which the O_2-content of the arterial blood was lowered to the same degree by means of carbon monoxide (ASMUSSEN and CHIODI 1941). While the carbon monoxide hypoxemia differs from the low oxygen hypoxemia with respect to the arterial oxygen tension the two conditions are similar in that a peripheral hypoxia exists in both cases. It is, therefore, reasonable to assume, that the pulse acceleration at least partly is called forth by a changed distribution of the blood due to the hypoxic peripheral vasodilatation. The mechanism by which such a pulse acceleration is called forth is probably the same as that which causes the pulse acceleration occurring when the blood distribution is changed on assumption of an upright body position. As discussed elsewhere (ASMUSSEN and NIELSEN 1955) it is possible that this pulse acceleration is elicited from stretch receptors in the walls of the heart (cf. JARISCH and ZOTTERMAN 1948, SCHAEFER 1950 and PAINTAL 1953) whereas the assumption that it is called forth via the arterial baroceptors hardly can be maintained, as there is no sign of a diminuation of the arterial blood pressure in any of the above mentioned conditions. In low oxygen it must further be assumed that the lowered arterial pO_2 via the arterial chemoreceptors plays a rôle for the pulse acceleration as it is a well established fact that a low pO_2 stimulates the chemoreceptors and thereby can call forth a pulse acceleration. A low arterial O_2 content without a fall in arterial O_2 tension (as in CO poisoning) has no stimulating effect on the chemoreceptors (SCHMIDT and COMROE 1940, DUKE, GREEN and NEIL 1952). The fact that the stroke volume, especially during work, is considerably lower in CO poisoning than in normal conditions (ASMUSSEN and CHIODI 1941) whereas the stroke volume in low oxygen is but slightly

2.

Experiments in pure oxygen					
Number of experiments	O_2-uptake l/min	Cardiac output l/min	a-v O_2-diff. cc/l	Pulse rate	Stroke volume cc
10	—	17.4 ± 0.33	125	139	125
13	2.49	18.8 ± 0.47	133	151	125
10	2.87	21.7 ± 0.43	133	161	132
4	3.28	22.2	147	168	132
2	3.44	24.9	139	158	158

diminished (table 1) may be explained by the stimuli set up in the chemoreceptors in the latter case.

The relatively slight effect that a lowering of the arterial pO_2 and O_2 saturation (down to about 35 mm Hg and 65 to 70 per cent, respectively) has on the cardiac output makes it highly improbable that the small degree of arterial unsaturation that occurs during muscular work should play any significant rôle for the regulation of the cardiac output in exercise. This view is further confirmed by the fact that abolition of this arterial unsaturation during work by means of oxygen breathing has no measurable effect on the size of the cardiac output (table 2).

In heavy work during which the conditions in the working muscles are more or less anaerobic it has been found that oxygen breathing lowers the lung ventilation considerably and it has been suggested (ASMUSSEN and NIELSEN 1946) that the relatively high lung ventilation in heavy work is caused by anaerobic metabolites. The production of these metabolites can be eliminated or reduced by oxygen breathing, whereby the found decrease in lung ventilation may be explained. The present experiments which also were performed during very heavy work in which partly anaerobic conditions must have prevailed (table 2) show that oxygen breathing did not have such an effect on the cardiac output as it has on the lung ventilation. Therefore, there is no reason to assume that anaerobic metabolites play any part in the regulation of the size of the cardiac output during work.

The other problem which it was intended to investigate, viz. the effect of oxygen breathing on oxygen uptake and cardiac output in maximum work in which it earlier has been found that the former can be increased considerably above what is possible in air, had to be left unsolved, because the fixed position of the sub-

jects necessitated by the technique for the cardiac output determinations made it impossible for the subjects to attain a high enough work intensity.

Summary.

The cardiac output in rest and work in low oxygen and during work in pure oxygen was determined by means of the dye injection method.

In low oxygen (arterial oxygen saturation 65 to 70 per cent) it was found that the cardiac output in rest and work was increased 10 to 20 per cent above the values from control experiments in air. The increase was due to a pulse acceleration as the stroke volume was slightly decreased.

In pure oxygen no measurable effects on the cardiac output during work was found. The pulse rate decreased 4 to 8 per cent.

The results are discussed with a special view to the possible mechanisms by which the cardiac output is regulated during muscular work.

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Note on the Effect of X-rays and Hormones on the Resorption Rate of Injected $NaH^{14}CO_3$.

By

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Received 28 May 1955.

In a previous communication (FORSSBERG and HEVESY, 1955) evidence was presented for an effect of exposure to X-rays on the rate of resorption of injected ^{14}C-labelled sodium bicarbonate as indicated by the rate of exhalation of $^{14}CO_2$. The circulating bicarbonate in the body of the mouse has a mean life-time of a few minutes only and consequently any change in the resorption rate will be indicated by a corresponding change in the amount of exhaled $^{14}CO_2$ within the first minutes after the injection of the labelled bicarbonate.

The percentage $^{14}CO_2$ exhaled by irradiated mice in the first 4 minutes following injection was found to be 29 % of the amount administered which compares with 45 % exhaled by the non-irradiated controls. In a later stage of the experiment as in the 6—10 min. time interval the amount of $^{14}CO_2$ exhaled by the exposed animals was found to be larger than that exhaled by the controls as the early preferential exhalation by the controls lead to an accumulation of $^{14}CO_2$ by the exposed mice.

An X-ray effect did not manifest itself when administering the bicarbonate by intravenous injection. As the effect observed may have been mediated through hormonal action on the resorption process we investigated the effect of both adrenaline and ATCH-administration on $^{14}CO_2$ exhalation. Subcutaneous injection of adrenaline was found in our previous investigation to

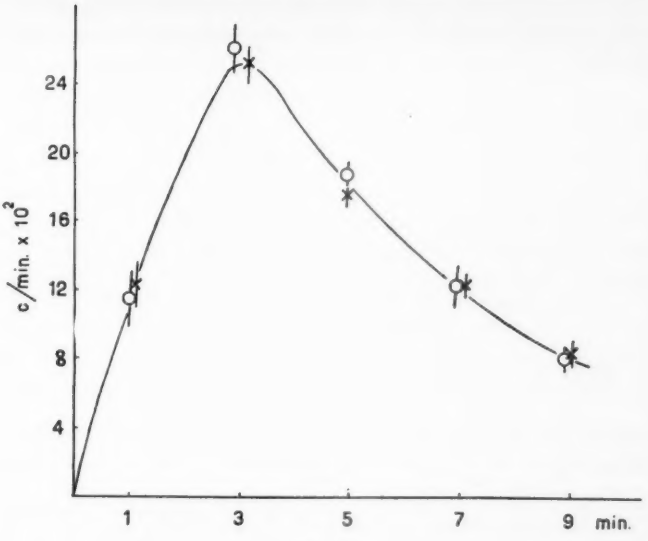

Fig. 1.

decrease the rate of exhalation of $^{14}CO_2$ in a similar way as did irradiation whereas injection of ACTH increased the immediate output of $^{14}CO_2$. Experiments in which exposure to radiation and hormonal treatment were combined brought out that an ACTH injection given shortly before irradiation annihilates the effect of the latter. The last mentioned observation can possibly be explained by an immediate depressive effect of X-irradiation on the resorption of $NaH^{14}CO_3$ due to a momentary blockage of the release of ACTH. The combined effect of an adrenaline injection and irradiation had the unexpected effect that the initial output of $^{14}CO_2$ was much higher than that from control mice although each agent alone caused a depressed $^{14}CO_2$ exhalation. The mechanism inherent in this synergistic action is obscure.

When following up these experiments half a year later — the experimental arrangement being the same as in the foregoing paper — we found irradiation with 2,000 r not to influence the exhalation of $^{14}CO_2$ which follows injection of bicarbonate (Fig. 1). Concomitantly a shift in the reaction of the animals against adrenaline had occurred. In the previous investigation 2 γ adrenaline, given subcutaneously 15 min. before the injection of the

Table 1.

Hormone	Amount of subcutaneously injected hormone	Change in initial exhalation of $^{14}CO_2$ caused by hormone injection %	Significance test for the diff. between control and treated animals		
			n	t	p
Adrenaline	2 γ, 15 min. before $NaH^{14}CO_3$[1]	+ 23.9	14	2.09	0.05
»	10 γ, 15 min. before $NaH^{14}CO_3$	— 25.9	19	1.93	0.05
ACTH	On the two days prior to experiment 0.008 I. U. On day of experiment 0.04 I. U. 10 min. before $NaH^{14}CO_3$[1]	+ 24.8	25	2.08	0.05

[1] Similar to the procedure in the previous investigation.

labelled bicarbonate, was found to decrease the $^{14}CO_2$-output by about 30 % in contrast to an increased output of 24 % found in the present investigation (Table 1).

An adrenaline dose increased to 10 γ resulted, however, in a reaction which was qualitatively and quantitatively similar to that observed in our earlier investigation after administration of 2 γ. Likewise, after increasing the X-ray dose to 4,000—5,000 r a similar initial depression of the resorption rate was observed as was found after 2,000 r in the first investigation. The influence of the ACTH-treatment was quantitatively the same at both the two occasions.

No definite opinion can be expressed concerning the reason for the change in the sensitivity of mice to exposure and to adrenaline administration; whether it is based on seasonal variations (the first investigation was performed in winter—spring, this second one in the autumn) or is due to a genetical shift in the mouse strain (the animals were heterozygots, although from the same farm). It is to be emphasized that a shift occurred both in the response to exposure to X-rays as well as to adrenaline administration. This observation suggests the existence of some correlation between the response of the body to hormonal changes and the effect of X-rays as far as the resorption and distribution of labelled compounds is concerned. There remains considerable uncertainty concerning the mechanism of the interaction particularly because the sensitivity to ACTH-treatment was found to be quantitatively similar in the two instances whereas the X-ray

and adrenaline effect were disimilar. These findings have, however, a general implication in isotope and other studies as rapid resorption and distribution processes in the body may likely be influenced by the hormonal balance of the animal.

Summary.

Exposure to X-rays, administration of adrenaline or ACTH were found to influence the rate of resorption of injected labelled bicarbonate by mice as indicated by the amount of $^{14}CO_2$ exhaled. When repeating the experiments half a year later 2—3 times as high X-ray dose and a 5 times higher adrenaline dose had to be applied to obtain the same effect on $^{14}CO_2$ exhalation while no change in the sensitivity to ACTH was observed.

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On the Mechanism of Formation of the Glomerular Fluid.

By

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It is generally assumed that the glomerular fluid, *i. e.* the primary urine, is formed from plasma by way of a *filtration* process across the glomerular membrane, the driving force being the algebraic sum of the gradients of the hydraulic and "osmotic" pressures across the capillary wall. This concept has been criticized by CHINARD (1952 a and b, cf. also PAPPENHEIMER, 1953), who maintains that only a process of *diffusion* is compatible with experimental data. The distinction between filtration and diffusion is important in the formulation of the rate equation for the process. In a forthcoming paper (GARBY and WALLENIUS, to be published) the concept of filtration was used.

A formulation of the rate equation for the glomerular fluid flow is of interest not only because it gives information as to the mechanism of the flow, but also because it contains various parameters which determine the flow. This is essentially the approach made by CHINARD (1952 a and b), who rightly states that Starling's well known law was derived on purely intuitive grounds.

The present paper is a discussion of the mechanism of formation of the glomerular fluid in terms of conventional physicochemical principles. It is proposed that the term filtration should be retained for the description of the process.

Theoretical Considerations.

A rigorous treatment of the transport processes which occur in the system may be given by the application of the thermodynamics of irreversible processes (see for instance DE GROOT, 1952, and HAASE, 1952).

The following system will be considered. A membrane, the glomerular wall, is surrounded by two liquid phases, plasma and primary urine. The two phases may differ in composition and pressure but are assumed to have the same temperature. If, within the phases, differences in temperature, pressure and composition are smoothed out quickly compared to the rate of exchange of matter and energy between the phases, the system fulfills the condition of being discontinuous from the standpoint of the present treatment. It is sufficient to regard only two components in each phase: water and protein. We have then

$$J_1 = L_{11}X_1 + L_{12}X_2 \quad (1)$$
$$J_2 = L_{21}X_1 + L_{22}X_2 \quad (2)$$

where J_1 and J_2 denote the flux of water and protein molecules respectively (mass per unit area and time). The different L:s are phenomenological coefficients relating the fluxes to the "forces" X, which are given by

$$X_1 = \Delta\mu_1 \quad (3)$$
$$X_2 = \Delta\mu_2 \quad (4)$$

where $\Delta\mu$ is the difference in chemical potential between the two phases.

Although the Onsager reciprocity relation states that $L_{12} = L_{21}$ and although the "forces" X are known, nothing can be learned from the equations as regards the magnitude of the fluxes J_1 and J_2. Without a detailed knowledge of the structure of the membrane, the phenomenological coefficients cannot be evaluated. They do not contain any conventional diffusion coefficients.

We may now turn to the conditions within the membrane and ask whether we may obtain any information on the flow of the different species by the phenomenological-thermodynamic treatment. The system is now a continuous one, *i. e.* the intensive properties are dependent not only on the time but also on the space coordinates. For such a system we have

$$\vec{J}_i = \sum_{k=1}^{N-1} L_{ik} \, (\vec{X}_k - \vec{X}_N) \quad (i = 1,2,\ldots\ldots,N-1) \quad (5)$$

for isothermal conditions. The driving "forces" are here given by

$$\vec{X}_i = -(\text{grad } \mu_i)_T \quad (6)$$

if external forces (gravitation, centrifugal fields etc.) are neglected. J_i is the diffusion flux of i defined with respect to the velocity of the mass centre v, so that

$$\vec{J}_i = \varrho_i \, (\vec{u}_i - \vec{v}) \quad (7)$$

where ϱ_i is the density of i and u_i is the mean velocity of the particles of species i.

In this case there exists a simple relationship between the L_{ik}:s and the conventional diffusion coefficients. However, as far as the fluxes of the components across a fixed membrane is concerned, no information can be obtained from the treatment without making assumptions about the mass flow velocity, which, of course, is dependent on the membrane structure.

The following proposition is now made: whenever, in a system like that considered above, the velocity of the mass centre is different from zero, the mechanism of the transport of the fluid as a whole is *filtration*. Any liquid, subject to mass flow through a porous membrane, will pass this filter according to an expression formally identical with equations (1) and (2). The phenomenological coefficients in an actual case depend on the character of the membrane and in some cases also on the nature of the driving "forces". However, for the same membrane, the coefficients are the same whether hydraulic or "osmotic" pressures are involved. (The term "osmotic" pressure is defined here in exactly the same way as done by CHINARD (1952 b).) The equivalency between hydraulic and "osmotic" pressures has been established many times (MONTONNA and JILK, 1941, KUHN, 1951, and CLAESSON and JACOBSSON, 1954). If the conditions required by a discontinuous system are not fulfilled, we must expect deviations from the theory (cf. KUHN, 1951). This complication is of no importance for the present argumentations, but enters into every application of the theory and may have some bearing on recent work on fluid transport across capillaries (PAPPENHEIMER, 1953, BOJESEN, 1954). The important thing is that we must expect the same mechanism of transport to occur whether we have hydraulic or "osmotic" pressures operating.

In conformity with the above, the term *diffusion*, when referring to net solvent flow, should be restricted to transport across such frames of reference that do not move with respect to the mass centre.

From a molecular point of view, it is obvious that the distinction between filtration and diffusion looses its meaning when the "pore" dimensions of the filter approach those of a single water molecule and where the molecules pass the membrane without mutual internaction (cp. ONSAGER, 1945).

Discussion.

In the special case under consideration, the passage of fluid across the glomerular membrane, it is obvious that the velocity of the mass centre is different from zero. Hence, no information can be obtained from the treatment given above without making any assumptions about the membrane structure. Indeed, the mobility term to be used in the rate equation for water transport is not given by any defined physical constant. There are, however, reasons to believe that a reasonable approximation of it can be given by applying the Hagen-Poiseuille law in a special way (cf. RENKIN, 1954). This is done by GARBY and WALLENIUS (to be published), giving results with respect to various parameters of the glomerular membrane which are at variance with those calculated by CHINARD (1952 b), who maintains that diffusion is the mechanism by which fluid passes the glomerular filter. CHINARD argues that since the condition of no hydrostatic pressure difference does not give zero net flow, the mechanism must be one of diffusion. Accordingly, in the formulation of the rate equation for water transport (eq. (15) in CHINARD (1952 b)), the mobility for water transport is given by the use of the "free" diffusion coefficient of water in water and no consideration is taken of the mass flow. In the opinion of the present author and as shown above, such a calculation is not justified.

Summary.

The mechanism of the formation of the glomerular fluid is discussed in terms of conventional physico-chemical principles.

A recent formulation of the rate equation for this process is criticized.

A definition of the term filtration is proposed, and it is shown that this term may conveniently be used for the description of the mechanism of formation of the glomerular fluid.

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INDEX

Pag.

A. Lundberg, The Electrophysiology of the Submaxillary Gland of the Cat . . . 1

B. Westin, Oxygen and Carbon Dioxide Tension in the Umbilical Vessels of the Human Fetus in Prolonged Asphyxia 26

C.-J. Clemedson, H. Ison Hultman and B. Sörbo, A Combination of Rhodanese and Ethanethiosulfonate as an Antidote in Experimental Cyanide Poisoning . . 31

I. Bergström, G. Magnusson, E. Odeblad and D. Ziliotto, A Study on the Uptake of Injected Radioiron in the Mouse Kidney 36

K.-B. Augustinsson, The Normal Variation of Human Blood Cholinesterase Activity 40

A. Carlsson and B. Lindquist, Comparison of Intestinal and Skeletal Effects of Vitamin D in Relation to Dosage 53

G. C. H. Bauer, A. Carlsson and B. Lindquist, A Comparative Study on the Metabolism of Sr^{90} and Ca^{45} 56

G. C. H. Bauer, A. Carlsson and B. Lindquist, Some Properties of the Exchangeable Bone Calcium 67

E. Asmussen and M. Nielsen, The Cardiac Output in Rest and Work at Low and High Oxygen Pressures 73

A. Forssberg and G. Hevesy, Note on the Effect of X-rays and Hormones on the Resorption Rate of Injected $NaH^{14}CO_3$ 84

L. Garby, On the Mechanism of Formation of the Glomerular Fluid 88

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Pag.
1
26
31
36
40
53
56
67
73
84
88